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(54) Title: DENDRITIC CELL RECEPTOR

(57) Abstract

The invention provides isolated human DEC-205, its extracellular domain and functionally equivalent fragments thereof. Also provided are polynucleotides encoding same and vectors which include such polynucleotides. Further provided are methods of recombinantly producing human DEC-205, an extracellular domain thereof or a functionally equivalent fragment, and ligands that bind to human DEC-205 or a fragment thereof. Also provided are constructs for use in prophylaxis or therapy comprising such a ligand, human DEC-205 or an extracellular domain thereof coupled to a toxin or to an antigen capable of inducing a protective immune response in a patient.

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DENDRITIC CELL RECEPTOR

FIELD OF THE INVENTION

This invention relates to dendritic cell receptors. In particular, it relates to human DEC-205, to the production and use thereof, and to ligands which bind to it. Human DEC-205 and its ligands are useful in prophylaxis and therapy.

BACKGROUND OF THE INVENTION

Dendritic cells perform important immunoregulatory functions by presenting antigens in the form of peptides bound to cell-surface major histocompatibility complex (MHC) molecules to T cells. Identification of the mechanism by which this antigen presentation function is achieved therefore has important implications in manipulating immune response in prophylaxis and therapy, particularly in humans.

Jiang *et al*, *Nature* 375: 151-155 (1995) disclose a murine dendritic cell receptor having a molecular weight of 205 kDa (murine DEC-205). However, they do not disclose a receptor on human dendritic cells.

The applicant has now identified a receptor on human dendritic cells. It is broadly to this receptor (likely to be the human homolog of murine DEC-205) that the present invention is directed.

SUMMARY OF THE INVENTION

The present invention has a number of aspects. In a first aspect, the invention provides isolated human DEC-205 which has an approximate molecular weight of 198-205 kDa and which includes the following amino acid sequences:

(i) TVDCNDNQPGAICYYSGNETEKEVKPVDSVKCPSPVLNTPWIPF
QNCCYNFIITKNRHMATTQDEVQSTCEKLHPKSHILSIRDEKE
NNFVLEQLLYFNYMASWVMLGITYRNNSL; and

(ii) SQHRLFHLHSQKCLGLDITKSVNELRMFSCDSSAML;

or a functionally equivalent fragment thereof.

In a further aspect, the invention provides isolated human DEC-205 which comprises the amino acid sequence shown in Figure 11 or a functionally equivalent fragment thereof.

In a still further aspect, the invention provides isolated mature human DEC-205, which comprises the amino acids 27 to 1722 shown for human DEC-205 in Figure 11.

In yet a further aspect, the invention provides an extracellular domain of human DEC-205 or a functionally-equivalent fragment thereof.

In a preferred embodiment, the extracellular domain fragment includes at least a portion of carbohydrate recognition domain (CRD7), spacer, and a portion of carbohydrate recognition domain (CRD8) of human DEC-205 (amino acids 1208 to 1323 of the amino acid sequence of Figure 11).

In a still further aspect, the invention provides a polynucleotide encoding human DEC-205 or its extracellular domain as defined above. This polynucleotide is preferably DNA, more preferably cDNA, but can also be RNA.

In a specific embodiment, the polynucleotide coding for human DEC-205 includes the following nucleotide sequences:

(iii) A ACA GTT GAT TGC AAT GAC AAT CAA CCA GGT GCT ATT TGC
TAC TAT TCA GGA AAT GAG ACT GAA AAA GAG GTC AAA CCA GTT
GAC AGT GTT AAA TGT CCA TCT CCT GTT CTA AAT ACT CCG TGG
ATA CCA TTT CAG AAC TGT TGC TAC AAT TTC ATA ATA ACA AAG
AAT AGG CAT ATG GCA ACA ACA CAG GAT GAA GTT CAT ACT AAA
TGC CAG AAA CTG AAT CCA AAA TCA CAT ATT CTG AGT ATT CGA
GAT GAA AAG GAG AAT AAC TTT GTT CTT GAG CAA CTG CTG TAC
TTC AAT TAT ATG GCT TCA TGG GTC ATG TTA GGA ATA ACT TAT
AGA AAT AAX TCT CTT; and

(iv) ATT AAT ATG CTG TGG AAG TGG GTG TCC CAG CAT CGG CTC TTT
CAT TTG CAC TCC CAA AAG TGC CTT GGC CTC GAT ATT ACC AAA
TCG GTA AAT GAG CTG AGA ATG TTC AGC TGT GAC TCC AGT GCC
ATG CTG TGG TGG AAA TGC GAG CAC CA

wherein X is T or G.

In a further embodiment, the polynucleotide comprises part or all of the nucleotide sequence of Figure 10.

In yet a further aspect, the invention provides a vector including a polynucleotide as defined above.

In still a further aspect, the invention provides a method of producing human DEC-205, the extracellular domain thereof or a functional fragment comprising the steps of:

(a) culturing a host cell which has been transformed or transfected with a vector as defined above to express the encoded human DEC-205, extracellular domain or fragment; and

(b) recovering the expressed human DEC-205, extracellular domain or fragment.

As yet an additional aspect, the invention provides a ligand that binds to human DEC-205 or its extracellular domain as defined above.

Preferably, the ligand is an antibody or antibody binding fragment or carbohydrate bearing protein.

The antibody or antibody binding fragment can be used in methods for extracting or isolating activated dendritic cells.

In still a further aspect, the invention provides a construct for use in therapy or prophylaxis. The construct will usually be a ligand-antigen construct or a DEC-205-antigen construct although ligand-toxin and DEC-205-toxin constructs are also contemplated. The ligand-antigen construct preferably consists of an antibody or antibody binding fragment which binds to human DEC-205 and a host-protective antigen. The DEC-205-antigen construct preferably consists of at least the extra-cellular domain of human DEC-205 and a host-protective antigen.

In yet further aspects, the invention contemplates methods of therapy or prophylaxis which employ human DEC-205, ligands or constructs containing them.

In yet a further aspect, the invention provides a molecule (hapten) which may be used to generate antibodies for identifying or purifying human dendritic cells, which includes a peptide based upon part or all of the sequence of Figure 11.

DESCRIPTION OF THE DRAWINGS

While the invention is broadly as defined above, it will be appreciated by those persons skilled in this art that it is not limited thereto and that it includes embodiments more particularly described below. It will also be better understood by reference to the accompanying drawings, in which

Figure 1 shows the structure of human DEC-205;

Figure 2 shows the strategy for isolation of human DEC-205 cDNA.
A. A schematic presentation of human DEC-205 mRNA with the regions corresponding to DEC-205 domains. The positions of the primers used for the cDNA cloning and analysis are indicated with arrows. The positions of reverse transcriptase-polymerase chain reaction (RT-PCR) fragments 1 to 6 and the clone pBK14-1 are indicated with bars. B. RT-PCR amplification of fragment 1 and 2 from L428 and HEL cell line RNA. L428 and HEL cells were subjected to RT-PCR with two pairs of degenerate primers (DEC-a/-b, and DEC-d/-e), fractionated by electrophoresis through 2% agarose gel, and stained with ethidium bromide. C. RT-PCR and 3'-RACE amplification of fragment 3 and 4 from L428 cells using the primers 028/023 and 029/019, respectively. A cDNA pool of L428 cells was subjected to 3'-RACE and RT-PCR, electrophoresed through 0.8% agarose gel, and stained with ethidium bromide. The numbers on the top correspond to the name of fragment in Fig. 2A. The positions of DNA molecular size standard are indicateds to the right. The estimated molecular size of the RT-PCR products are indicated to the left;

Figure 3 shows protein similarity between human and mouse DEC-205.
A. The predicted amino acid sequence of human DEC-205 is aligned with the mouse homolog. The regions corresponding to DEC-205 domain structure are bracketed. The positions of amino acids are shaded where there are identical or conservatively replaced amino acids between the sequences, and the asterisks indicates conserved cysteines. The diamonds indicates potential N-glycosylation sites conserved between the sequences. The arrow indicates one amino acid deletion in CRD-5 of human DEC-205. The circles

indicate conserved potential serine-phosphorylation sites by protein kinase C (open circle) or casein kinase (closed circle). B. The % identity between human and mouse DEC-205 is indicated above each domain (boxed, See Fig. 2A for key);

Figure 4 shows that human DEC-205 is probably a one-copy gene. Genomic DNA isolated from the peripheral blood of four individuals was digested with the restriction enzymes BglII, BamHI, HindIII or EcoRI and subjected to Southern blot analysis with the [^{32}P]cysteine-rich domain probe. The final wash was 0.3 x SSC at 65°C. The positions of the DNA molecular size standards are indicated to the right;

Figure 5 shows that human DEC-205 gene localizes on chromosome 2.
A somatic cell hybrid panel blot (restriction-digested with PstI) was subjected to Southern blot analysis with the [^{32}P]cysteine-rich domain probe. The final wash was 0.3 x SSC at 65°C. The positions of the DNA molecular size standards are indicated to the right. The estimated molecular size of the probe-specific bands are indicated to the left. The asterisk indicates weakly hybridized bands. M, male; F, female;

Figure 6 shows that human DEC-205 gene maps to chromosome band 2q24. A. A metaphase spread of human chromosomes were subjected to fluorescent in situ hybridization (FISH) with 6.6 kb human DEC-205 cDNA probe. The final wash was 0.1 x SSC at 60°C. The FISH image was overlaid with a DAPI-stained chromosome image. The DEC-205 specific signals are indicated by the arrowheads. B. An inverted image of chromosome 2 containing DEC-205-specific signal (see Fig. 6A) is aligned with an ideogram of chromosome 2. The chromosome band corresponding to DEC-205 gene is indicated to the right;

Figure 7 shows that expression of DEC-205 transcripts within human hematopoetic cell lines. Total RNA prepared from the cell lines were subjected to Northern blot analysis with the [^{32}P]fragment 3 (A and B), or [^{32}P]-actin (C) probes. The final wash was 0.1 x SSC at 65°C. The positions of the RNA molecular size standards are indicated to the right. The estimated molecular size of DEC-205 transcripts are indicated to the left. A, 24 h exposure; B, 72 h exposure;

Figure 8 shows RT-PCR analysis of DEC-205 mRNA in human DC preparations. Specific product is seen using lineage negative; fresh DC (lane 2) and a stronger signal

with CMRF-44$^+$ low density cultured DC (lane 3). CD8$^+$ T lymphocytes (lane 1) contain no DEC-205 mRNA Ethidium stain.

Figure 9 represents the result of an ELISA assay showing a monoclonal antibody binding specifically to DEC-205 peptide 1 and not peptide 3. Positive control binding of a hyperimmunized rabbit anti-DEC-205-peptide 1 serum and hyperimmunized rabbit anti-DEC-205-peptide 2 serum are shown;

Figure 10 gives the DNA sequence for human DEC-205 (coding region only);

Figure 11 gives the human DEC-205 amino acid sequence.

DETAILED DESCRIPTION OF THE INVENTION

A. Human DEC-205

The human DEC-205 of the invention is believed to be the human homolog of murine DEC-205 and has an approximate molecular weight of 198 to 205 kDa. It has the structure shown in Figures 1 and 2A. It also has the deduced amino acid sequence shown in Figure 11.

Human DEC-205 can usefully be provided in a number of different forms. These include human DEC-205 itself, the "mature" form of human DEC-205, and the extracellular receptor domain of human DEC-205.

The "mature" form of human DEC-205 of the invention is human DEC-205 less its native amino-terminus leader or signal sequence, whereas the extracellular receptor domain is human DEC-205 lacking the signal sequence, the transmembrane region and cytoplasmic domain (where present).

The extracellular domain may be identified through commonly recognised criteria of extracellular amino acid sequences. The determination of appropriate criteria is known to those skilled in the art, and has been described, for example by Hopp et al., Proc. Natl. Acad. Sci. USA 78, 3824-3828 (1991); Kyte et al., J. Mol. Biol. 157, 105-132 (1982); Emini, J. Virol 55, 836-839 (1985); Jameson et al. CA BIOS 4, 181-186 (1988); and Karplus et al. Naturwissenschaften 72, 212-213 (1985). Amino acid domains predicted by these criteria to be surface exposed are characteristic of extracellular domains.

The amino acid sequences of the predicted regions for human DEC-205 are shown in Figure 3A. These include the amino acid sequences for the signal peptide, cysteine-rich domain, fibronectin type II domain, Carbohydrate Recognition Domain-1, (CRD-1), CRD-2, CRD-3, CRD-4, CRD-5. CRD-6, CRD-7, CRD-8, CRD-9, CRD-10, transmembrane domain and cytoplasmic domain.

Human DEC-205 of the invention or its extracellular receptor domain (or parts thereof) may be prepared by methods known in the art. Such methods include protein synthesis from individual amino acids as described by Stuart and Young in "Solid Phase Peptide Synthesis", Second Edition, Pierce Chemical Company (1984). It is however preferred that human DEC-205 and/or its extracellular receptor domain or parts thereof be prepared by recombinant methods as will be detailed hereinafter.

Example 1 provides further details of human DEC-205.

Example 1

Langerhans cells were prepared from human skin. Epidermal cell suspensions were prepared from split thickness normal human breast skin by 30 min dispase (Boehringer-Mannheim, Mannheim, Germany; 0.5% in PBS) treatment at 37°C, followed by 10 min disaggregation in the presence of trypsin (0.25% in PBS), DNase I (5U/ml in PBS) and 5mM EDTA at room temperature. Langerhans cells were then enriched by Ficoll/Metrizoate gradient separation (d=1.077g/cm^3). Final cell suspensions contained 3-15% Langerhans cells as determined by HLA-DR positivity. Total RNA was extracted using Trizol reagent according to the manufacturer's instructions.

Degenerate primers were prepared on an Applied Biosystems DNA Synthesizer with the primer sequences (d) and (e) as set out below:

(d) 5'-GAX ACY GAX GGY TTX TGG AA-3'
(e) 3'-GCY GTX TTZ TCZ AAC CAC AT-5'

wherein X is C or T, Y is A, C, G or T, and Z is G or A.

Single stranded cDNA was prepared using total RNA and reverse transcribed by AMV reverse transcriptase using the 3' primer (e). Subsequently, the cDNA was amplified

using the 5'(d) and 3'(e) primer using PCR amplification according to techniques known in the art.

The amplified products were run on a 2% agarose gel and visualized with ethidium bromide staining.

The DNA was purified and ligated into the T tailed pGEM vector (available from Promega) using standard techniques. The ligation mixture was transformed into competent *E. coli* JM 109 bacteria (available from Promega) which were grown on agar plates with appropriate antibiotic selection. Two colonies were isolated. DNA was prepared and digested with restriction enzymes. Two inserts of the same size as the PCR product were sequenced by double-stranded DNA sequencing techniques using a Sequence Kit (Sequence 2.0 USB Lab Supply, Pierce). The two clones corresponded to human DEC-205.

The amino acid sequence of human DEC-205 was determined to include the following amino acid sequences:

(i) TVDCNDNQPGAICYYSGNETEKEVKPVDSVKCPSPVLNTPWIPF
QNCCYNFIITKNRHMATTQDEVQSTCEKLHPKSHILSIRDEKE
NNFVLEQLLYFNYMASWVMLGITYRNNSL; and

(ii) SQHRLFHLHSQKCLGLDITKSVNELRMFSCDSSAML.

Determination of these sequences was fundamental to isolating the cDNA for human DEC-205 detailed below.

In the partial sequences given above, individual amino acids are represented by the single letter code as follows:

Amino Acid	Three-letter abbreviation	One-letter symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Asparagine or aspartic acid	Asx	B
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic Acid	Glu	E
Glutamine or glutamic acid	Glx	Z
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Unidentified		X

This code also applies to the predicted full sequence of Figure 11, deduced from the cDNA encoding human DEC-205 isolated as described below.

B. Polynucleotides Encoding Human DEC-205

In another aspect of this invention, the applicants provide polynucleotides encoding human DEC-205 or its extracellular domain. These polynucleotides may be DNA (isolated from nature, synthesised or cDNA) or RNA. Most often, the polynucleotides will be DNA.

The polynucleotides of the invention specifically include those which include the nucleotides

(iii) A ACA GTT GAT TGC AAT GAC AAT CAA CCA GGT GCT ATT TGC
TAC TAT TCA GGA AAT GAG ACT GAA AAA GAG GTC AAA CCA GTT
GAC AGT GTT AAA TGT CCA TCT CCT GTT CTA AAT ACT CCG TGG
ATA CCA TTT CAG AAC TGT TGC TAC AAT TTC ATA ATA ACA AAG
AAT AGG CAT ATG GCA ACA ACA CAG GAT GAA GTT CAT ACT AAA
TGC CAG AAA CTG AAT CCA AAA TCA CAT ATT CTG AGT ATT CGA
GAT GAA AAG GAG AAT AAC TTT GTT CTT GAG CAA CTG CTG TAC
TTC AAT TAT ATG GCT TCA TGG GTC ATG TTA GGA ATA ACT TAT
AGA AAT AAX TCT CTT; and

(iv) ATT AAT ATG CTG TGG AAG TGG GTG TCC CAG CAT CGG CTC TTT
CAT TTG CAC TCC CAA AAG TGC CTT GGC CTC GAT ATT ACC AAA
TCG GTA AAT GAG CTG AGA ATG TTC AGC TGT GAC TCC AGT GCC
ATG CTG TGG TGG AAA TGC GAG CAC CA

wherein X is T or G,
as well as the full nucleotide sequence shown in Figure 10,
but are not limited thereto.

The invention also includes within its scope functional equivalents of these polynucleotides.

This aspect of the invention will now be illustrated by the following Examples.

EXAMPLE 2
EXPERIMENTAL PROCEDURES

Cell culture—The cell lines, HEL, K562, KG-1, THP-1, U937, Mann and Jurkat were obtained from the American Type Culture Collection (Rockville, MD). L428 cells were provided by V. Diehl (Klinik for Innere Medizin, Cologne, Germany). HDLM2 and KMH2 cells were obtained from the German Collection of Micro-organisms and Cell Culture (Braunscfweig, Germany). Mono Mac 6 cells (Bufler *et al* (1995) *Eur. J. Immunol.* 25, 604-610) were provided by H. Engelmann (Institute for Immunology, Munchen, Germany). All cell lines were maintained in RPMI 1640, 10% fetal calf

serum, 100 U/ml penicillin, 100 ug/ml streptomycin except that HDLM2 cells were with 20% fetal calf serum.

Isolation of leukocytes---Leukocyte populations were isolated using standard laboratory procedures.

Isolation of cDNA encoding for human DEC-205---A set of degenerate oligonucleotide primers were designed based on the published amino acid sequence of mouse DEC-205 (Jiang *et al* (1995), above) and synthesized in house or by Life Technologies (Auckland, New Zealand) (see Fig. 2A). These primers were:
DEC-a (5'-AAYATGCTNTGGAARTGGGT-3'),
DEC-b (5'-TGRTGYTCRCAYTTCCACCA-3'),
DEC-d (5'-GAYACNGAYGGNTTYTGGAA-3') and
DEC-e (5'-GCNGTYTTRTCRAACCACAT-3'),
where Y=C or T, R= A or G, N=A or C or G or T. Total RNA isolated from L428 or HEL cells was reverse transcribed with avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI) at 55°C for 1 h using the primers DEC-b or DEC-e. PCR was performed using the resultant cDNA and Taq polymerase (Boehringer Mannheim, Auckland, New Zealand) with the primers DEC-a/-b for DEC-b-primed or DEC-d/-e for DEC-e-primed cDNAs. The PCR conditions used were the initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 54°C for 1 min, extension at 72°C for 1 min, and the final extension at 72°C for 5 min. The PCR reactions were fractionated with 2% agarose gel in 40 mM Tris-acetate, pH 8.3, 1 mM EDTA (TAE) buffer, and stained with 0.5 ug/ml ethidium bromide. The PCR fragments (fragment 1 and 2, see Fig. 2A and 2B) were cloned into pGEM-T vector (Promega), and sequenced manually using Sequenase DNA sequencing kit (Amersham Life Science, Auckland, New Zealand).

A set of oligonucleotide primers nested within the DNA sequence of fragment 1 and 2 were synthesized (see Fig. 2A). These primers were:
023 (5'-GCTCTAGAAACATGACCCATGAAGCC-3' containing a XbaI site),
028 (5'-GCTCTAGACATCGGCTCTTTCATTTGT-3' containing a XbaI site) and
029 (5'-CGGGATTCACAGTTGATTGCAATGACA-3' containing a EcoRI site)
where incorporated restriction sites are underlined. Two ug of poly(A) RNA from L428 cells was reverse transcribed with 200 U of SuperScriptII (LifeTechnoloies) at 45°C for 1 h using an oligo d(T) adaptor primer

018 (5'-GACTAGTCTGCAGAATTCTTTTTTTTTTTTTTTT-3', containing a SpeI, PstI, and EcoRI sites). After heat-inactivation at 70°C for 15 min, the reaction was incubated with 1 U RNaseH (Life Technologies) at 37°C for 30 min, heat-inactivated at 70°C for 15 min, and diluted to 1 ml with 10 mM Tris-HCl, pH 8.0,1 mM EDTA (L428 cDNA pool). In order to isolate the fragment 3 (connecting the fragment 1 and 2) (see Fig. 2A), PCR was performed with 5 ul of L428 cDNA pool, the primers 028 and 023, and 2.5 U of Expand enzyme mix (Boehringer Mannheim). The PCR conditions were the initial denaturation at 94°C for 2 min, 10 cycles of 10 cycles of denaturation at 94°C for 15 sec, annealing at 53 °C for 30 sec, and extension at 68°C for 4 min, followed by 20 cycles of denaturation at 94°C for 15 sec, annealing at 53°C for 30 sec, and extension at 68°C for 4 min plus additional 20 sec for each cycle, and the final extension at 68°C for 15 min. 3'-rapid amplification of cDNA ends (3'-RACE) (Frohman *et al* (1988) *Proc. Natl. Acad. Sci. USA* 85, 8998-9002) was performed in order to isolate the fragment 4 (connecting the fragment 1 and the 3'-untranslated region of DEC-205) (see Fig. 2A). PCR was performed with 5 ul of L428 cDNA pool and the primer 029 and an adaptor primer 019 (5'-GACTAGTCTGCAGAATTC, containing a SpeI, PstI and EcoRI site), in the same conditions for the fragment 3. The PCR reactions were fractionated with 0.8% agarose gel in TAE buffer, and stained with ethidium bromide. Both the fragment 3 and 4 were restriction digested with XbaI and EcoRI, respectively, and cloned into pBluescript II (Stratagene, La Jolla, CA). The representative clones from the fragment 3 (pB38f1) and 4 (pb30-3) were sequenced with a LI-COR automated sequencer (LI-COR, Lincoln, Nebraska) using SequiTherm cycle sequecing kit (Epicentre Technologies, Madison, WI). If required, these plasmids were subjected to exonucleaseIII-nested deletion using Erase-A-Base system (Promega), and used for sequencing.

An oligo dT-primed L428 cDNA library was prepared using ZAP Express cDNA Gigapack Cloning kit (Stratagene) according to manufacturer's instruction. The fragment 3 was labeled with [α-32P]dCTP (NEN) using Multiprime system (Amersham Life Science). The library was screened by plaque hybridization with the [^{32}P]fragment 3 using standard techniques (Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2Ed., Cold Spring Harbour Laboratory, New York, USA). The specific activity of the probe was 0.8 x10^9 cpm/ug DNA and used at 1 x 10^6 cpm/ml. The final wash was in 0.1 x SSC, 0.5% SDS at 65°C (1 x SSC is 0.15 M NaCl, 15 mMM Na-citrate, pH7.0). Positive clones were converted to phagemid pBK-CMV (Stratagene) and sequenced using an automated sequencer.

In order to verify the DNA sequence obtained from the PCR clones, pB38f for fragment 3 and pB30-3 for fragment 4, the fragment 5 was PCR-amplified from a L428 cDNA pool using primers 058 (5'-CGGGATCCCTCTGGCCGCGCACTAATGA-3' containing a BamHI site) and 050 (5'-CCGCTCGAGCTGTGGATACCAGCACATGCCT-3' containing a XhoI site) (see Fig. 2A). The PCR conditions were identical to that for the fragment 3 except using longer extension period (6 min) for cycling. The fragment 5 was sequenced directly using the IRD_{40}-labeled custom primers (MWG-Biotech, Ebersberg, Germany) and a LI-COR automated sequencer without cloning. These primers were: IRD001 (5'-GATGGGAACTCTTATGGGAGACCT-3' at nucleotide 523-555), IRD002 (5'-TGATGCAGGCTGGCTGCCAAATAA-3' at nucleotide 1134-1157), IRD003 (5'-AACTGGGCAACTGTTGGTGGAAGA-3' at nucleotide 1759-1782), IRD004 (5'-ATGGCGAAGAGGCTGGCATTTCTA-3' at nucleotide 2334-2357), IRD005 (5'-CTCAAGCAAGCGATACCTGTCACT-3' at nucleotide 2972-2995), IRD006 (5'-TGGGCAACTCGAAGACTGTGTAGT-3' at nucleotide 3624-3647), IRD007 (5'-CACCAGCACAGCATTCTTGCTTGT-3' at nucleotide 4168-4191) and IRD008 (5'-ATTTGTGAGCAGACTGATGAGGGA-3' at nucleotide 4797-4820). The sequences of these primers were based on those of pb38f1 and pb30-3, and they were positioned as 540-650 bp apart, ensuring the generation of contigs overlapping by at least 100 bp after automated sequencing.

Southern blot analysis---Genomic DNA was prepared from peripheral blood of patients with hematological disorders (each patient was karyotyped at Canterbury Health Laboratories, Christchurch, New Zealand). Approximately 8 ug of genomic DNA was digested with BglII, BamHI, EcoRI, or HindIII, fractionated in 0.8% agarose gel in 89 mM Tris-borate, pH 8.3, 2 mM EDTA, and transfered to Hybond N+ by capillary reaction. A PCR-fragment corresponding to the cyteine-rich domain was PCR-amplified from pBK14-1 using the primers 058 and 059 (5'-CGGAATTCGATCTCATGATAAGGCTGGTCACA-3' containing a EcoRI site) (see Fig. 2A). Briefly, PCR was performed with 2 ng of pBK14-1, the primer 058 and 059, and Taq polymerase. The PCR conditions used were the initial denaturation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 15 sec, annealing at 55°C for 15 sec, extension at 72°C for 30 sec, and the final extension at 72°C for 5 min. The 450 bp PCR product was labeled with [α-^{32}P]dCTP using Multiprime labeling system (Amersham Life Science). The blot was hybridized with the probe using standard technique (Sambrook *et al*, (1989), above). The specific activity of the probe was 0.8 x 10^9 cpm/ug

DNA and used at 1 x 10^6 cpm/ml. The final wash was in 0.3 x SSC, 0.5% SDS at 65°C, and exposed to X-OMAT AR film (Kodak) with an intensifying screen at -70°C.

A blot containing PstI-digested genomic DNA from a human-rodent somatic hybrid cell panel was obtained from Oncor (Gaithersburg, MD), and probed with the [^{32}P]cysteine-rich domain fragment as described above.

Fluorescent in situ hybridization---Metaphase spreads were prepared from phytohaemagluttinin-stimulated peripheral blood lymphocytes of a 46,XY male donor using standard cytogenetic procedures. The fragment 6 was amplified by recombinant PCR with the fragment 3 and 4 (see Fig. 2A). PCR was performed with each of the fragment 3 and 4 and the primers 028 and 019 in the same conditions for the fragment 3 except using longer extension period (7 min) for cycling. The fragment 6 was labelled with biotin-14-dCTP using a BioPrime random prime labelling kit (Bethesda Research Laboratories, Gathersberg, Maryland), and hybridized to metaphase cells on slides. Conditions for hybridization and immunofluorescent detection were essentially as described (Morris *et al*, (1993) *Human Genetics*, 91, 31-36), except that Cot 1 suppression was not required, slides were washed to a stringency of 0.1xSSC, 60°C after hybridization, and an additional amplification step was needed because of the small size of the probe. For precise chromosome band localization, DAPI and FITC images were captured separately for each metaphase from the fluorescent microscope to computer using a Photometrics KAF1400 CCD camera and IPLAB Spectrum software (Signal Analytics, VA), and colour-joined using Multiprobe extension software.

Northern blot analysis---Approximately 10 ug of total RNA from cultured cells were fractionated in formaldehyde-denatured 1% agarose gel and transferred to Hybond N+ (Amersham) using 3 M NaCl, 8 mM NaOH, 2 mM sarkosyl with Turboblotter (Schleicher & Schuell, Keene, NH) for 3 h. The membrane was UV-crosslinked (Stratalinker, Stratagene), and hybridized with [^{32}P]fragment 3 or [^{32}P]human §-actin probe using standard techniques (Sambrook *et al* (1989), above). The specific activity of the probes were 0.9-1.1 x 10^9 cpm/ug DNA and used at 0.7-1.1 x 10^6 cpm/ml. The final wash was in 0.1 x SSC, 0.5% SDS at 68°C, and exposed to X-OMAT AR film (Kodak) with intensifying screen at -70°C.

Reverse transcription-PCR analysis---Total RNA from isolated leukocytes was incubated with RNase-free DNaseI (Life Technologies), and was reverse transcribed

using SuperscriptII with the oligo dT adaptor primer 018. PCR was performed using a pair of DEC-205 specific primers 060 (GTGGATCCAGTACAAGGGTCA at nucleotide 4655-4686) and 056 (ACCAAATCAGTCCGCCCATGA at nucleotide 5116-5096) with Taq polymerase in the presence of a PCR additive, Q buffer (Qiagen) by touch down PCR (Don, R.H., Cox, P.T., Wainwright, B.J., Baker, K., and Mattick, J.S., (1991) *Nucleic Acid Res.* 19, 4008). PCR conditions used were the initial denaturation at 92°C for 2 min, 21 cycles of denaturation at 92°C for 15 sec, annealing at 60°C minus 0.5°C/cycle for 15 sec, extension at 68°C for 30 sec, 15 cycles of denaturation at 92°C, annealing at 50°C, extention at 68°C for 1 min and the final extension at 68°C for 5 min. Human glycelaldehyde-3-phosphate dehydrogenase (GAPDH) (Tokunaga, K., Nakamura, Y., Sakata, K., Fujimori, K., Ohkubo, M., Sawada, K., and Sakiyama, S. (1987) *Cancer Res.* 47, 5616-5619) was used for normalization. The primers for GAPDH were 053 (ATGGGGAAGGTGAAGGTCGGA-3' at nucleotide 61-81), and 055 (AGGGGCCATCCACAGTCTTCT-3' at nucleotide 634-614). The PCR reactions were fractionated with 1.5 % agarose gel in TAE buffer, and stained with 0.5 ug/ml ethidium bromide.

Sequence data analysis---The National Center of Biotechnology Information (NCBI) Center electronic mail server BLAST was used to search for homologous sequences. Sequence alignments and motif search were done using Bestfit and Motifs programs, respectively, of GCG computer package (Madison, WI).

RESULTS

Isolation of cDNA for human DEC-205. Based on the amino acid sequence of mouse DEC-205, a set of degenerate primers were synthesized and used to perform RT-PCR using the Hodgkin's disease-derived L428 cell line and the myeloid HEL cell lines (Fig. 2). The two pair of primers (DEC-d/-e, and DEC-a/-b) gave rise to the specific RT-PCR products, fragment 1 (390 bp) and 2 (150 bp), respectively (Fig. 2A and 2B). These specific fragments were cloned and sequenced (data not shown). The deduced amino acid sequences of fragment 1 and 2 were ~80% identical to that of mouse DEC-205, indicating that these fragments were derived from the cDNA of human DEC-205.

Primers nested within these fragments were synthesized and further RT-PCR and 3'-RACE performed using a L428 cDNA pool reverse transcribed with an oligo dT adapter primer 018. A 3.8 kb RT-PCR product (fragment 3) was obtained using primer 028 and

023 (Fig. 2A and 2C). A 3.2 kb 3'-RACE product (fragment 4) was obtained using primer 029 and an adaptor primer 019 (Fig. 2A and 2C). The fragment 3 was cloned and several identical clones were identified by restriction enzyme map analysis (data not shown), and one of which, pb38f1, was fully sequenced: The DNA sequence of the fragment 3 (pB38f1) extending from the middle of cysteine-rich domain to the middle of CRD-8 (Fig. 2A), was 82% identical to the published mouse DEC-205 cDNA sequence. The fragment 4 was cloned and two distinct clones identified by restriction enzyme map analysis. Both clones were partially sequenced and the 3' end DNA sequence of one clone (eg. pb30-3) was found to contain a poly A tail, and with 72% identical to 3'-untranslated region of mouse DEC-205 (data not shown). Therefore, the pb30-3 was sequenced to obtain the DNA sequence of the coding region of DEC-205 plus partial 3'-untranslated region. The resulting DNA sequence for the coding region was ~80% identical to that of mouse DEC-205 spanning from the middle of CRD-8 to the end of cytoplasmic domain (Fig. 2A). The DNA sequences obtained from pb38f1 and pb30-3 overlapped by 320 bp, covering 95% of human DEC-205 coding region.

In order to complete the 5' end of the DEC-205 cDNA sequences a L428 cDNA library was screened by plaque hybridization using ^{32}P-labeled fragment 3 as a probe. A clone (pBK14-1) was isolated, and the 1.5 kb insert of this clone was sequenced (Fig. 2A). The sequence was ~80% identical to the mouse sequence and corresponded to the signal peptide, cysteine-rich domain, fibronectin type II domain, CRD-1 and part of the CRD-2. The pBK14-1 contained 51 bp 5'-untranslated region, and overlapped with fragment 3 by ~1.2 kb.

To validate the DNA sequence obtained from the PCR clones, a further RT-PCR fragment (fragment 5) amplified with primers 058 (nested in the cysteine-rich domain) and 050 (located ~130 bp downstream of the stop codon) was prepared (Fig. 2A). The fragment 5 PCR product was sequenced directly using IRD_{41}-labeled custom primers without cloning. A total of 10 point mutations, presumably generated because of the low fidelity of thermostable polymerases were found and corrected in the PCR clone-derived DNA sequence. The complete cDNA sequence for human DEC-205 is 5166 bp in size, and encodes for a predicted 198 kDa type I transmembrane protein with 1722 amino acids before post translational modification.

The deduced amino acid sequence of human DEC-205 showed 77% overall identity with the homologous mouse protein (Fig. 3A). All the cysteines, and putative N-glycosylation

sites in the extracellular domain of mouse DEC-205, were conserved in the human sequence. In the cytoplasmic domain the putative serine phosphorylation sites by protein kinase C or casein kinase, and a tyrosine, which appears to be important for coated pit-mediated internalization (Ezekowitz, R.A.B., Sastry, K., Bailly, P., and Warner, A. (1990) *J. Exp. Med.* 172, 1785-1794; and Zvaritch, E., Lambeau, G., and Lazdunski, M. (1996) *J. Biol. Chem.* 271, 250-257), were also conserved. There was one amino acid deletion within the CRD-5 in human DEC-205. All the extracelluar domains, including the cysteine-rich domain, fibronectin type II domain, and CRD1-10 were 74-87% identical between human and mouse sequences (Fig. 3B), suggesting the importance of these domains for the function of DEC-205. In contrast, the two hydrophobic domains, including the signal peptide and transmembrane domain, showed much lower identity (57% and 52%, respectively (Fig. 3B)) with the mouse protein, confirming the observation that these hydrohobic domains are more variable, and rapidly evolved structures (Von Heijne, G. (1990) *J. Membrane Biol.* 115, 195-201).

DEC-205 is a single copy gene with polymorphism---Peripheral blood-derived genomic DNA from 4 individuals was restriction enzyme-digested with BglII, BamHI, HindIII or EcoRI, and subjected to Southern blot analysis. The cysteine-rich domain of the macrophage mannose receptor (Kim, S.J., Ruiz, N., Bezouska, K., and Drickamer, K. (1992) *Genomics* 14, 721-727; and Harris, N., Peters, L.L., Eicher, E.M., Rits, M., Raspberry, D., Eichbaum, Q.G., Super, M., and Ezekowitz, R.A.B. (1994) *Biochem. Biophys. Res. Com.* 198, 682-692) and phospholipase A2 receptor (Ancian, P., Lambeau, G., Mattei, M.G., and Lazdunski, M. (1995) 270, 8963-8970) is encoded by one exon. Therefore, we amplified the cysteine-rich domain of human DEC-205 using primers 058 and 059 as a potential single exon probe (450 bp), and used this to probe the Southern blot in high stringency. A single band appeared in BglII-, BamHI- or HindIII-digested genomic DNA from all individuals, indicating that DEC-205 is a single copy gene (Fig. 4). The EcoRI digests, however, produced a single band in two individuals and double bands in another, indicating that the DEC-205 gene is polymorphic. Further Southern blot analysis with larger panel of individuals showed identical results (data not shown). Therefore, DEC-205 is a single copy gene with at least one polymorphic site.

DEC-205 gene maps to chromosome band 2q24---In order to map the human DEC-205 gene, a somatic cell hybrid panel Southern blot (PstI-digested) was probed with the [^{32}P]cysteine-rich domain as described above (Fig.5). A 3.0 kb band in human genomic DNA was found to hybridize strongly, and the identical band appeared in chromosome

2-containing somatic human-mouse hybrid cells, indicating that DEC-205 gene localizes on chromosome 2. The probe also hybridized weakly with hamster DNA, suggesting the presence of DEC-205 homolog in hamster as well as in the mouse (which also hybridized strongly). The origin of the weakly hybridized bands with apparent polymorphism in the human DNA-containing lanes is not known. The identical band appeared in chromosome 2, and may either be related to an alternative exon structure for this region of DEC-205 or result from weak cross hybridization to another gene on chromosome 2.

Fluorescent in situ hybridization then was used to map the DEC-205 gene in detail (Fig. 6A and 6B). The 6.4 kb recombinant PCR fragment (fragment 6) (Fig. 2A) was prepared from fragment 3 and 4, labeled with biotinylated nucleotides, and used as a probe in a high stringency (Fig. 6A). Ninety-one (80%) of a combined total 114 metaphase cells analysed from three experiments showed fluorescent signals on one (27) or both (64) chromosomes 2 in the middle of the long arm, specifically in band q24 (Fig. 6B). High resolution banding analysis provided a more precise location of signals (not shown). No additional site-specific signals were detected on any other chromosome.

DEC-205 exhibits multiple transcripts in cell lines---A panel of human cell lines, including myeloid, B lymphoid, T lymphoid and Hodgkin's desease-derived cell lines, were analyzed for the expression of DEC-205 transcripts by Northern blot analysis with the [^{32}P]fragment 3 as a probe (Fig. 7A and 7B). Two DEC-205 transcripts, 7.8 and 9.5 kb in size, were detected, and the 7.8 kb transcript was the most abundant. The expression level varied between cell lines, however the myeloid cell line THP-1, the B lymphoid cell line Mann and the Hodgkin's desease cell line KMH2 showed the highest level of expression. Even with longer exposure, DEC-205 transcripts were not detectable in K562, KG-1, Monomac and Jurkat cells, suggesting these cells are DEC-205 negative (Fig. 7B). Interestingly all Hodgkin's disease-derived cell lines tested express the transcripts. Semiquantitative RT-PCR studies also support these results (data not shown).

C. Recombinant Expression of Human DEC-205

In yet another aspect, the present invention relates to the recombinant expression of human DEC-205 or of its extracellular domain.

The Polynucleotides that encode human DEC-205 or the extracellular domain of the invention may be inserted into known vectors for use in standard recombinant DNA

techniques. Standard recombinant DNA techniques are those such as are described in Sambrook et al.; "Molecular Cloning" 2nd Edition Cold Spring Harbour Laboratory Press (1987) and by Ausubel et al., Eds, "Current Protocols in Molecular Biology" Greene Publishing Associates and Wiley-Interscience, New York (1987).

Vectors for expressing proteins in bacteria, especially E. coli, are known. Such vectors include the PATH vectors described by Dieckmann and Tzagoloff in J. Biol. Chem. 260, 1513-1520 (1985). These vectors contain DNA sequences that encode anthranilate synthetase (TrpE) followed by a polylinker at the carboxy terminus. Other expression vector systems are based on beta-galactosidase (pGEX); lambda P maltose binding protein (pMAL); and gluthathione S-transferase (pGST) - see Gene 67, 31 (1988) and Peptide Research 3, 167 (1990).

Vectors useful in yeast and insect cells are available and well known. A suitable example of a yeast vector is the 2μ plasmid.

Suitable vectors for use in mammalian cells are also known. Such vectors include well-known derivatives of SV-40, adenovirus, retrovirus-derived DNA sequences and vectors derived from combination of plasmids and phage DNA.

Further eucaryotic expression vectors are known in the art (e.g. P.J. Southern and P. Berg, J. Mol. Appl. Genet. 1, 327-341 (1982); S. Subramani et al, Mol. Cell. Biol. 1, 854-864 (1981); R.J. Kaufmann and P.A. Sharp, "Amplification And Expression of Sequences Cotransfected with a Modular Dihydrofolate Reductase Complementary DNA Gene," J. Mol. Biol. 159, 601-621 (1982); R.J. Kaufmann and P.A. Sharp, Mol. Cell. Biol. 159, 601-664 (1982); S.I. Scahill et al, "Expression And Characterization Of The Product Of A Human Immune Interferon DNA Gene In Chinese Hamster Ovary Cells," Proc. Natl. Acad. Sci. USA 80, 4654-4659 (1983); G. Urlaub and L.A. Chasin, Proc. Natl. Acad. Sci. USA 77, 4216-4220, (1980).

The expression vectors useful in the present invention contain at least one expression control sequence that is operatively linked to the DNA sequence or fragment to be expressed. The control sequence is inserted in the vector in order to control and to regulate the expression of the cloned DNA sequence. Examples of useful expression control sequences are the lac system, the trp system, the tac system, the trc system, major operator and promoter regions of phage lambda, the control region of fd coat protein, the

glycolytic promoters of yeast, e.g. the promoter for 3-phosphoglycerate kinase, the promoters of yeast acid phosphatase, e.g. Pho5, the promoters of the yeast alpha-mating factors, and promoters derived from polyoma, adenovirus, retrovirus, and simian virus, e.g. the early and late promoters or SV40, and other sequences known to control the expression of genes of prokaryotic and eucaryotic cells and their viruses or combinations thereof.

Vectors containing the receptor-encoding DNA and control signals are inserted into a host cell for expression of the receptor. Some useful expression host cells include well-known prokaryotic and eucaryotic cells. Some suitable prokaryotic hosts include, for example, E. coli, such as E. coli SG-936, E. coli HB 101, E. coli W3110, E. coli X1776, E. coli X2282, E. coli DHT, and E. coli MR01, Pseudomonas, Bacillus, such as Bacillus subtilis, and Streptomyces. Suitable eucaryotic cells include yeast and other fungi, insect, animal cells, such as COS cells and CHO cells, human cells and plant cells in tissue culture.

D. Ligands

The invention also includes ligands that bind to human DEC-205 of the invention.

The ligand will usually be an antibody or an antibody binding fragment raised against human DEC-205 or its extracellular domain, or against fragments thereof.

Such antibodies may be polyclonal but are preferably monoclonal. Monoclonal antibodies may be produced by methods known in the art. These methods include the immunological method described by Kohler and Milstein in Nature 256, 495-497 (1975) and Campbell in "Monoclonal Antibody Technology, the Production and Characterization of Rodent and Human Hybridomas" in Burdon et al. Eds, Laboratory Techniques in Biochemistry and Molecular Biology, Volume 13, Elsevier Science Publishers, Amsterdam (1985); as well as by the recombinant DNA method described by Huse et al. in Science 246, 1275-1281 (1989).

In yet another form, the ligand may also be a non-protein, probably carbohydrate containing, molecule that acts as a ligand when it binds to, or otherwise comes into contact with, human DEC-205.

In addition, ligands may be of two functional types. The first functional type of ligand is a molecule which binds to human DEC-205 and stimulates it in performing its normal function (a "stimulant ligand"). The second functional type of ligand is a molecule which binds to human DEC-205 and inhibits or prevents it performing its normal function (an "antagonistic ligand").

Both types of ligand will find application in either therapeutic or prophylactic treatments as described below.

Example 3 describes the production of anti-DEC-205 antibodies.

EXAMPLE 3

Production of Anti-DEC-205 Antibodies

A BALB/c mouse was immunized ip/sc with L428 cells and boosted SC with two peptides derived from the DEC-205 cDNA sequence. DEC-205 peptide 1 ATTQDEVHTKC (aa1267-aa1277) and DEC-205-peptide 2 TEKEVKPVDSVKC (aa1227-aa1239) were synthesized by Chiron Mimotopes Pty Ltd (Clayton, Victoria, Australia). After a third immunization with the two DEC-205 peptides sc/ip/IV the mouse was sacrificed and a spleen cell suspension prepared. The spleen cells were fused with the NS-1 myeloma cell line using standard techniques (Hock *et al*, Immunology 1994;83:573). A hybridoma was subsequently isolated, 2F5, which produced monoclonal antibody binding to the DEC-205-peptide 1 but not the DEC-205-peptide 2 or a third control DEC-205-peptide 3 (KCLGLDITKSVNELR) (aa82-aa96). This is shown by Figure 9.

E. Constructs

The invention also provides constructs. The constructs will generally include antigens against which an immune response is desired but can also include other products to be delivered specifically to dendritic cells. Toxins, such as the ricin A chain are not excluded. The other component of the construct will vary, being either a ligand as described above or at least the extracellular domain of human DEC-205. Both constructs will have the potential to manipulate the immune system of the host.

In the ligand-antigen constructs, ligands which bind to human DEC-205 (usually antibodies, antibody-binding fragments or carbohydrates expressing proteins) can be coupled or otherwise associated with the antigen against which an immune response is

desired. An example of such antigens are sugar-coated antigens such as tumour-associated antigens. In use, the ligand component binds to human DEC-205 and the dendritic cell is 'primed' with the associated antigen. This 'priming' action will assist in the induction of an immediate immune response against the antigen.

The ligand-antigen construct can take any appropriate form for administration to the dendritic cells. Such forms may differ depending upon whether the therapeutic protocol involves isolation of the patients dendritic cells (so that the priming action can take place *in vitro*) or whether the construct is to be administered to a patient *in vivo*.

The construct can be directly administered to a patient for *in vivo* treatment. It can also be administered in a form which allows the construct to be expressed within the patient.

One example of such a form for administration to a patient *in vivo* is a live recombinant viral vaccine. Such a vaccine includes a polynucleotide encoding the DEC-205 ligand (or a portion thereof) and the antigen. The vaccine is administered to the patient and, once within the patient, expresses the encoded ligand and antigen to bind to the patients dendritic cells (via human DEC-205).

A number of such live recombinant viral vaccine systems are known. An example of such a system is the *Vaccinia* virus system (US Patent 4603112; Brochier *et al.*, *Nature* 354:520 (1991)).

Administration can be via intravenous, intramuscular, subcutaneous, topical, oral, intra nasal, rectal or intracerebroventricular routes, as appropriate.

F. Applications

Human DEC-205, its ligands and the constructs discussed above can be employed therapeutically or prophylactically in accordance with this invention to promote or inhibit any of the known actions of dendritic cells and/or to manipulate the immune system.

Thus, the antagonistic ligands *per se* have potential application *inter alia* blocking or inhibiting the immune response during transplantation procedures.

Ligands also have application in delivering other products with which they are associated directly to dendritic cells. This can be for therapeutic purposes (where the delivered product is an immunogenic antigen) as discussed above. It can also be to target a toxin (such as the ricin A-chain specifically to dendritic cells to selectively destroy them as part of an immunosuppressive process.

G. The Use of Human DEC-205 to Detect Dendritic Cells in Cell Suspensions on Tissues and to Purify Dendritic Cells

Monoclonal antibodies or other ligands binding to DEC-205 may be used to identify or isolate DC for scientific study or therapeutic application. For this application, the antibodies or ligands can be used in conjunction with conventional identification/separation systems. An example of such a system is the avidin-biotin immunoaffinity system available from Cell-Pro Inc, Washington, USA (see US 5,215,927, US 5,225,353, US 5,262,334 and US 5,240,856).

This system employs directly or indirectly a biotinylated monoclonal antibody directed against a target cell and a column containing immunobilized avidin and can be readily adapted to extract activated human dendritic cells, in this case from human peripheral blood, using the anti-DEC-205 antibody as follows:

1. A sample of human peripheral blood containing the human dendritic cells is mixed with biotinylated anti-DEC-205 antibody and incubated to allow formation of antibody/human DC complexes.
2. Following incubation, the mixture is introduced into a CellPro continuous-flow immunoadsorption column filled with avidin-coated beads, the strong affinity between biotin and avidin causing the biotin-coated antibodies (together with the human DC to which they have bound) to adhere to the avidin-coated beads.
3. After unwanted cells present in the mixture are washed away, captured activated human DC are removed from the column by gentle agitation and are available for use.

Variations on this theme using the anti-DEC-205 antibody as primary antibody (to bind to activated DC) and a biotinylated secondary antibody (to bind to the anti-DEC-205 antibody) can also be employed.

It will be appreciated that before admixture with the anti-DEC-205 antibody in accordance with the above protocol, the human peripheral blood sample should be treated to ensure that the DC the sample contains are activated. This can easily be achieved by, for example, overnight incubation of the sample.

H. Functional Equivalents

The invention includes functional equivalents of human DEC-205, extracellular domains and nucleic acid molecules described above.

Human DEC-205 and its extracellular domain are or include proteins. A protein is considered a functional equivalent of another protein for a specific function if the equivalent protein is immunologically cross-reactive with, and has the same function as, the original protein. The equivalent may, for example, be a fragment of the protein, or a substitution, addition or deletion mutant of the protein.

For example, it is possible to substitute amino acids in a sequence with equivalent amino acids using conventional techniques. Groups of amino acids known normally to be equivalent are:

(a) Ala(A) Ser(S) Thr(T) Pro(P) Gly(G);
(b) Asn(N) Asp(D) Glu(E) Gln(Q);
(c) His(H) Arg(R) Lys(K);
(d) Met(M) Leu(L) Ile(I) Val(V); and
(e) Phe(F) Tyr(Y) Trp(W).

Substitutions, additions and/or deletions in human DEC-205 may be made as long as the resulting equivalent protein is immunologically cross-reactive with, and have the same function as, the native human DEC-205.

The equivalent human DEC-205 will normally have substantially the same amino acid sequence as the native human DEC-205. An amino acid sequence that is substantially the same as another sequence, but that differs from the other sequence by means of one or more substitutions, additions and/or deletions is considered to be an equivalent sequence. Preferably, less than 25%, more preferably less than 10%, and most preferably less than 5% of the number of amino acid residues in the amino acid sequence of the native human DEC-205 are substituted for, added to, or deleted from.

Equivalent nucleic acid molecules include nucleic acid sequences that encode proteins equivalent to human DEC-205 as defined above. Equivalent nucleic acid molecules also include nucleic acid sequences that, due to the degeneracy of the nucleic acid code, differ from native nucleic acid sequences in ways that do not affect the corresponding amino acid sequences.

Those persons skilled in the art will of course appreciate that the above description is provided by way of example only and that the invention is limited only by the lawful scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(1) APPLICANT: DEREK NIGEL JOHN HART

(2) TITLE: DENDRITIC CELL RECEPTOR

(3) NUMBER OF SEQUENCES:

(5) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5"HD FLOPPY DISC

(B) COMPUTER: IBM PC COMPATIBLE

(C) OPERATING SYSTEM: MS-DOS

(D) SOFTWARE: WORDPERFECT 6.1 FOR WINDOWS

(2) INFORMATION FOR SEQUENCE ID NO. 1:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1722 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) TOPOLOGY: LINEAR

(2) MOLECULE TYPE: PROTEIN

(3) SEQUENCE DESCRIPTION: SEQ ID NO. 1:

1 MRTGWAHPSP PGGAPHAALL VLRSRGALWP RTNDPFTIVH GNTGKCIKPV

51 YGWIVADDCD ETEDKLWKWV SQHRLFHLHS QKCLGLDITK SVNELRMFSC

101 DSSAMLWWKC EHHSLYGAAR YWLALKDGHG TAISNASDVW KKGGSEESLC

151 DQPYHEIYTR DGNSYGRPCE FPFLIDGTWH HDCILDEDHS GPWCATTLNY

201 EYDRKWGICL KPENGCEDNW EKNEQFGSCY QFNTQTALSW KEAYVSCQNQ

251 GADLLSINSA AELTYLKEKE GIAKIFWIGL NQLYSARGWE WSDHKPLNFL

301 NWDPDRPSAP TIGGSSCARM DAESGLWQSF SCEAQLPYVC RKPLNNTVEL

351 TDVWTYSDTR CDAGWLPNNG FCYLLVNESN SWDKAHAKCK AFSSDLISIH

401 SLADVEVVVT KLHNEDIKEE VWIGLKNINI PTLFQWSDGT EVTLTYWDEN

451 EPNVPYNKTP NCVSYLGELG QWKVQSCEEK LKYVCKRKGE KLNDASSDKM

501 CPPDEGWKRH GETCYKIYED EVPFGTNCNL TITSRFEQEY LNDLMKKYDK

551 SLRKYFWTGL RDVDSCGEYN WATVGGRRRA VTFSNWNFLE PASPGGCVAM

601 STGKSVGKWE VKDCRSFKAL SICKKMSGPL GPEEASPKPD DPCPEGWQSF

651 PASLSCYKVF HAERIVRKRN WEEAERFCQA LGAHLSSFSH VDEIKEFLHF

701 LTDQFSGQHW LWIGLNKRSP DLQGSWQWSD RTPVSTIIMP NEFQQDYDIR

751 DCAAVKVFHR PWRRGWHFYD DREFIYLRPF ACDTKLEWVC QIPKGRTPKT

801 PDWYNPDRAG IHGPPLIIEG SEYWFVADLH LNYEEAVLYC ASNHSFLATI

851 TSFVGLKAIK NKIANISGDG QKWWIRISEW PIDDHFTYSR YPWHRFPVTF

901 GEECLYMSAK TWLIDLGKPT DCSTKLPFIC EKYNVSSLEK YSPDSAAKVQ

951 CSEQWIPFQN KCFLKIKPVS LTFSQASDTC HSYGGTLPSV LSQIEQDFIT

1001 SLLPDMEATL WIGLRWTAYE KINKWTDNRE LTYSNFHPLL VSGRLRIPEN

1051 FFEEESRYHC ALILNLQKSP FTGTWNFTSC SERHFVSLCQ KYSEVKSRQT

1101 LQNASETVKY LNNLYKIIPK TLTWHSAKRE CLKSNMQLVS ITDPYQQAFL

1151 SVQALLHNSS LWIGLFSQDD ELNFGWSDGK RLHFSRWAET NGQLEDCVVL

1201 DTDGFWKTVD CNDNQPGAIC YYSGNETEKE VKPVDSVKCP SPVLNTPWIP

1251 FQNCCYNFII TKNRHMATTQ DEVHTKCQKL NPKSHILSIR DEKENNFVLE

1301 QLLYFNYMAS WVMLGITYRN NSLMWFDKTP LSYTHWRAGR PTIKNEKFLA

1351 GLSTDGFWDI QTFKVIEEAV YFHQHSILAC KIEMVDYKEE HNTTLPQFMP

1401 YEDGIYSVIQ KKVTWYEALN MCSQSGGHLA SVHNQNGQLF LEDIVKRDGF

1451 PLWVGLSSHD GSESSFEWSD GSTFDYIPWK GQTSPGNCVL LDPKGTWKHE

1501 KCNSVKDGAI CYKPTKSKKL SRLTYSSRCP AAKENGSRWI QYKGHCYKSD

1551 QALHSFSEAK KLCSKHDHSA TIVSIKDEDE NKFVSRLMRE NNNITMRVWL

1601 GLSQHSVDQS WSWLDGSEVT FVKWENKSKS GVGRCSMLIA SNETWKKVEC

1651 EHGFGRVVCK VPLGPDYTAI AIIVATLSIL VLMGGLIWFL FQRHRLHLAG

1701 FSSVRYAQGV NEDEIMLPSF HD*

(2) INFORMATION FOR SEQUENCE ID NO. 2:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5166

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(2) MOLECULE TYPE: cDNA

(3) SEQUENCE DESCRIPTION: SEQ ID NO. 2:

1 ATGAGGACAG GCTGGGCGCA CCCCTCGCCG CCCGGCGGGG CTCCTCATGC

51 TGCTCTTCTG GTTCTTCGAT CTCGCGGAGC CCTCTGGCCG CGCACTAATG

101 ACCCCTTCAC CATCGTCCAT GGAAATACGG GCAAGTGCAT CAAGCCAGTG

151 TATGGCTGGA TAGTAGCAGA CGACTGTGAT GAAACTGAGG ACAAGTTATG

201 GAAGTGGGTG TCCCAGCATC GGCTCTTTCA TTTGCACTCC CAAAAGTGCC

251 TTGGCCTCGA TATTACCAAA TCGGTAAATG AGCTGAGAAT GTTCAGCTGT

301 GACTCCAGTG CCATGCTGTG GTGGAAATGT GAGCACCACT CTCTGTACGG

351 AGCTGCCCGG TACTGGCTGG CTCTGAAGGA TGGACATGGC ACAGCAATCT

401 CAAATGCATC TGATGTCTGG AAGAAAGGAG GCTCAGAGGA AAGCCTTTGT

451 GACCAGCCTT ATCATGAGAT CTATACCAGA GATGGGAACT CTTATGGGAG

501 ACCTTGTGAA TTTCCATTCT TAATTGATGG GACCTGGCAT CATGATTGCA

551 TTCTTGATGA AGATCATAGT GGGCCATGGT GTGCCACCAC CTTAAATTAT

601 GAATATGACC GAAAGTGGGG CATCTGCTTA AAGCCTGAAA ACGGTTGTGA

651 AGATAATTGG GAAAAGAACG AGCAGTTTGG AAGTTGCTAC CAATTTAATA

701 CTCAGACGGC TCTTTCTTGG AAAGAAGCTT ATGTTTCATG TCAGAATCAA

751 GGAGCTGATT TACTGAGCAT CAACAGTGCT GCTGAATTAA CTTACCTTAA

801 AGAAAAAGAA GGCATTGCTA AGATTTTCTG GATTGGTTTA AATCAGCTAT

851 ACTCTGCTAG AGGCTGGGAA TGGTCAGACC ACAAACCATT AAACTTTCTC

901 AACTGGGATC CAGACAGGCC CAGTGCACCT ACTATAGGTG GCTCCAGCTG

951 TGCAAGAATG GATGCTGAGT CTGGTCTGTG GCAGAGCTTT TCCTGTGAAG

1001 CTCAACTGCC CTATGTCTGC AGGAAACCAT TAAATAATAC AGTGGAGTTA

1051 ACAGATGTCT GGACATACTC AGATACCCGC TGTGATGCAG GCTGGCTGCC

1101 AAATAATGGA TTTTGCTATC TGCTGGTAAA TGAAAGTAAT TCCTGGGATA

1151 AGGCACATGC GAAATGCAAA GCCTTCAGTA GTGACCTAAT CAGCATTCAT

1201 TCTCTAGCAG ATGTGGAGGT GGTTGTCACA AAACTCCATA ATGAGGATAT

1251 CAAAGAAGAA GTGTGGATAG GCCTTAAGAA CATAAACATA CCAACTTTAT
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1351 GAGCCAAATG TTCCCTACAA TAAGACGCCC AACTGTGTTT CCTACTTAGG
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1901 AAGCATCCCC TAAGCCTGAT GACCCCTGTC CTGAAGGCTG GCAGAGTTTC
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2751 TAAACCAACA GACTGTAGTA CCAAGTTGCC CTTCATCTGT GAAAAATATA
2801 ATGTTTCTTC GTTAGAGAAA TACAGCCCAG ATTCTGCAGC TAAAGTGCAA
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2951 GTGGCACCCT TCCTTCAGTG TTGAGCCAGA TTGAACAAGA CTTTATTACA
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3151 TTTTTTGAGG AAGAGTCTCG CTACCACTGT GCCCTAATAC TCAACCTCCA

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3701 TTGACAGTGT TAAATGTCCA TCTCCTGTTC TAAATACTCC GTGGATACCA

3751 TTTCAGAACT GTTGCTACAA TTTCATAATA ACAAAGAATA GGCATATGGC

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3901 CAACTGCTGT ACTTCAATTA TATGGCTTCA TGGGTCATGT TAGGAATAAC

3951 TTATAGAAAT AATTCTCTTA TGTGGTTTGA TAAGACCCCA CTGTCATATA

4001 CACATTGGAG AGCAGGAAGA CCAACTATAA AAAATGAGAA GTTTTTGGCT

4051 GGTTTAAGTA CTGACGGCTT CTGGGATATT CAAACCTTTA AAGTTATTGA

4101 AGAAGCAGTT TATTTTCACC AGCACAGCAT TCTTGCTTGT AAAATTGAAA

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4251 AGCATTAAAC ATGTGTTCTC AAAGTGGAGG TCACTTGGCA AGCGTTCACA

4301 ACCAAAATGG CCAGCTCTTT CTGGAAGATA TTGTAAAACG TGATGGATTT

4351 CCACTATGGG TTGGGCTCTC AAGTCATGAT GGAAGTGAAT CAAGTTTTGA

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4651 CAGGCATTGC ACAGTTTTTC AGAGGCCAAA AAATTGTGTT CAAAACATGA

4701 TCACTCTGCA ACTATCGTTT CCATAAAAGA TGAAGATGAG AATAAATTTG

4751 TGAGCAGACT GATGAGGGAA AATAATAACA TTACCATGAG AGTTTGGCTT

4801 GGATTATCTC AACATTCTGT TGACCAGTCT TGGAGTTGGT TAGATGGATC

4851 AGAAGTGACA TTTGTCAAAT GGGAAAATAA AAGTAAGAGT GGTGTTGGAA

4901 GATGTAGCAT GTTGATAGCT TCAAATGAAA CTTGGAAAAA AGTTGAATGT

4951 GAACATGGTT TTGGAAGAGT TGTCTGCAAA GTGCCTCTGG GCCCTGATTA

5001 CACAGCAATA GCTATCATAG TTGCCACACT AAGTATCTTA GTTCTCATGG

5051 GCGGACTGAT TTGGTTCCTC TTCCAAAGGC ACCGTTTGCA CCTGGCGGGT

5101 TTCTCATCAG TTCGATATGC ACAAGGAGTG AATGAAGATG AGATTATGCT

5151 TCCTTCTTTC CATGACTAA

CLAIMS:

1. Isolated human DEC-205 which has an approximate molecular weight of 198-205 kDa and which includes the following amino acid sequences:

(i) TVDCNDNQPGAICYYSGNETEKEVKPVDSVKCPSPVLNTPWIPF QNCCYNFIITKNRHMATTQDEVQSTCEKLHPKSHILSIRDEKE NNFVLEQLLYFNYMASWVMLGITYRNNSL; and

(ii) SQHRLFHLHSQKCLGLDITKSVNELRMFSCDSSAML;

or a functionally equivalent fragment thereof.

2. Isolated human DEC-205 which comprises the amino acid sequence of Figure 11, or a functionally equivalent fragment thereof.

3. Isolated human DEC-205 in mature form which comprises amino acids 27 to 1722 of the amino acid sequence of Figure 11.

4. The extracellular domain of human DEC-205 or a functionally equivalent fragment thereof as claimed in claim 1.

5. The extracellular domain of human DEC-205 having an amino acid sequence which includes amino acids 27 to 1661 of Figure 11 or a functionally equivalent fragment thereof.

6. An extracellular domain fragment as claimed in claim 5 which includes amino acids 1208 to 1323 of amino acid sequence of Figure 11.

7. A polynucleotide encoding human DEC-205, its extracellular domain or a fragment thereof as defined in any one of claims 1 to 6.

8. A polynucleotide as claimed in claim 7 which includes the following nucleotide sequences:

(iii) A ACA GTT GAT TGC AAT GAC AAT CAA CCA GGT GCT ATT TGC
TAC TAT TCA GGA AAT GAG ACT GAA AAA GAG GTC AAA CCA GTT
GAC AGT GTT AAA TGT CCA TCT CCT GTT CTA AAT ACT CCG TGG
ATA CCA TTT CAG AAC TGT TGC TAC AAT TTC ATA ATA ACA AAG
AAT AGG CAT ATG GCA ACA ACA CAG GAT GAA GTT CAT ACT AAA
TGC CAG AAA CTG AAT CCA AAA TCA CAT ATT CTG AGT ATT CGA
GAT GAA AAG GAG AAT AAC TTT GTT CTT GAG CAA CTG CTG TAC
TTC AAT TAT ATG GCT TCA TGG GTC ATG TTA GGA ATA ACT TAT
AGA AAT AAX TCT CTT; and

(iv) ATT AAT ATG CTG TGG AAG TGG GTG TCC CAG CAT CGG CTC TTT
CAT TTG CAC TCC CAA AAG TGC CTT GGC CTC GAT ATT ACC AAA
TCG GTA AAT GAG CTG AGA ATG TTC AGC TGT GAC TCC AGT GCC
ATG CTG TGG TGG AAA TGC GAG CAC CA

wherein X is T or G.

9. A polynucleotide as claimed in claim 7 which comprises all of the nucleotide sequence of Figure 10.

10. A polynucleotide as claimed in claim 7 which comprises nucleotides 64 to 5166 of the nucleotide sequence of Figure 10.

11. A polynucleotide as claimed in any one of claims 7 to 10 which is DNA.

12. A vector which includes a polynucleotide as claimed in claim 12.

13. A method of producing human DEC-205, an extracellular domain thereof or a functionally equivalent fragment comprising the steps of:

(a) culturing a host cell which has been transformed or transfected with a vector as defined above to express the encoded human DEC-205, extracellular domain or fragment; and
(b) recovering the expressed human DEC-205, extracellular domain or fragment.

14. A ligand that binds to human DEC-205 or a fragment thereof as claimed in any one of claims 1 to 3.

15. A ligand that binds to an extracellular domain of human DEC-205 or fragment thereof as claimed in any one of claims 4 to 6.

16. A ligand as claimed in claim 14 or claim 15 which is an antibody, or antibody binding fragment.

17. A construct for use in prophylaxis or therapy comprising a ligand as claimed in any one of claims 14 to 16 coupled to an antigen capable of inducing protective immune response in a patient.

18. A construct for use in prophylaxis or therapy comprising a ligand as claimed in any one of claims 14 to 16 coupled to a toxin.

19. A construct for use in prophylaxis or therapy comprising human DEC-205 or an extracellular domain thereof as claimed in any one of claims 1 to 6 coupled to an antigen capable of inducing a protective immune response in a patient.

20. A construct for use in prophylaxis or therapy comprising human DEC-205 or an extracellular domain thereof as claimed in any one of claims 1 to 6 coupled to a toxin.

21. A method of prophylaxis or therapy which comprises administering to a patient in need of the same human DEC-205 as claimed in any one of claims 1 to 3, an extracellular domain as claimed in any one of claims 4 to 6, a ligand as claimed in any one of claims 14 to 16, or a construct as claimed in any one of claims 17 to 20.

22. A process for isolating activated dendritic cells expressing human DEC-205 on the surface thereof comprising the step of contacting a sample containing said cells with a ligand as claimed in claim 16, and isolating those cells to which the ligand has bound.

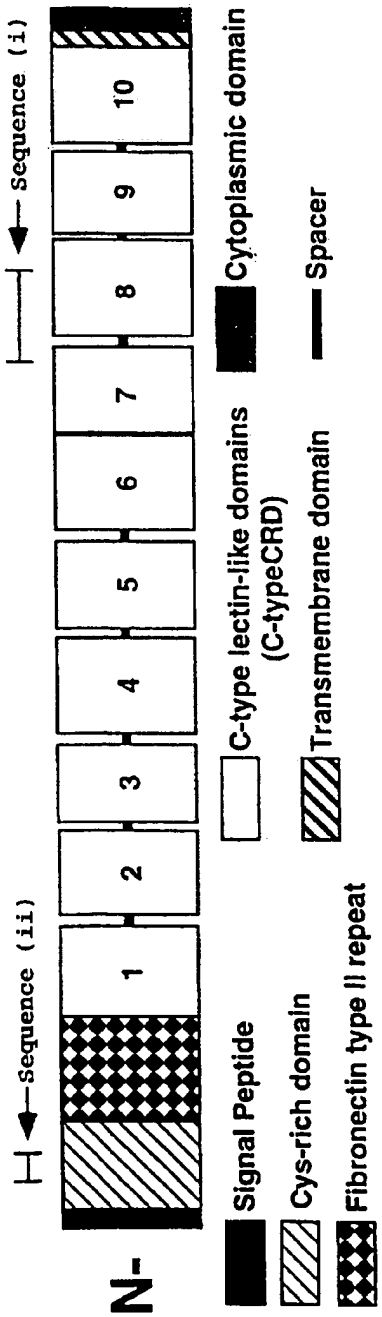
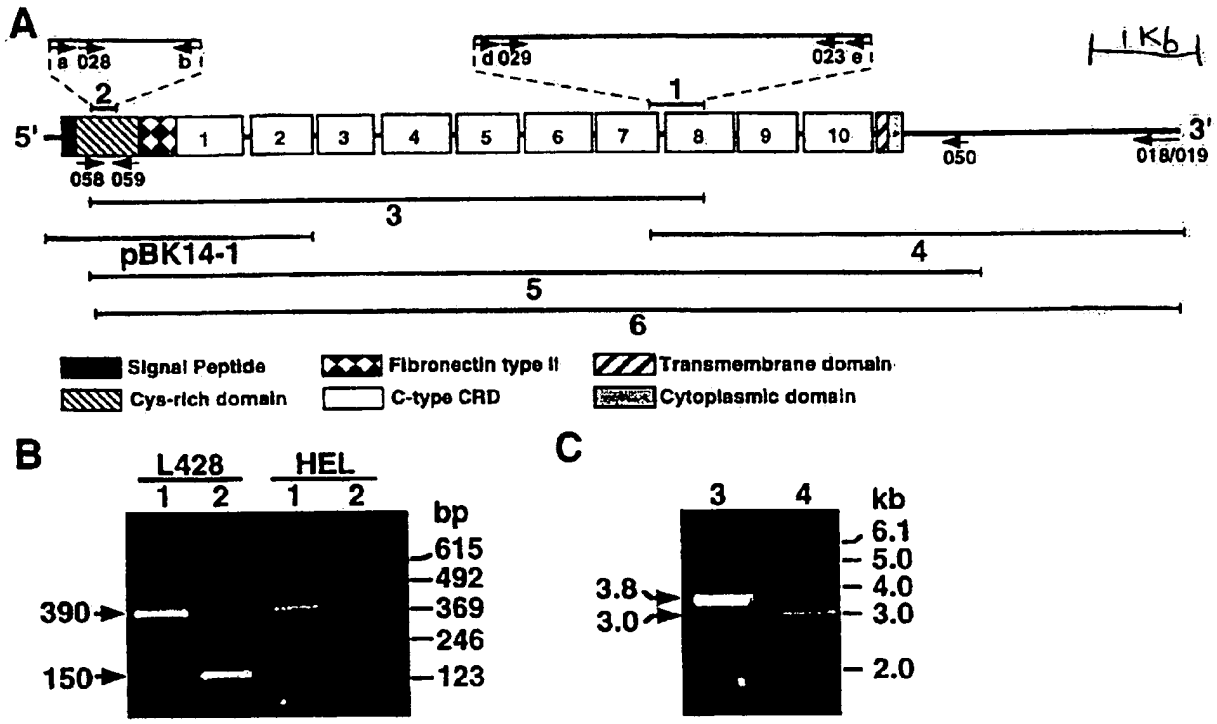

N-
Sequence (ii)
Sequence (i)
1
2
3
4
5
6
7
8
9
10
Signal Peptide
Cys-rich domain
Fibronectin type II repeat
C-type lectin-like domains (C-typeCRD)
Transmembrane domain
Cytoplasmic domain
Spacer

Figure 1


A
a 028
b
d 029
023 e
1Kb
2
1
5'
3'
1
2
3
4
5
6
7
8
9
10
058 059
050
018/019
3
pBK14-1
4
5
6
Signal Peptide
Fibronectin type II
Transmembrane domain
Cys-rich domain
C-type CRD
Cytoplasmic domain
B
L428
HEL
1 2 1 2
bp
615
492
369
246
123
390
150
C
3 4
kb
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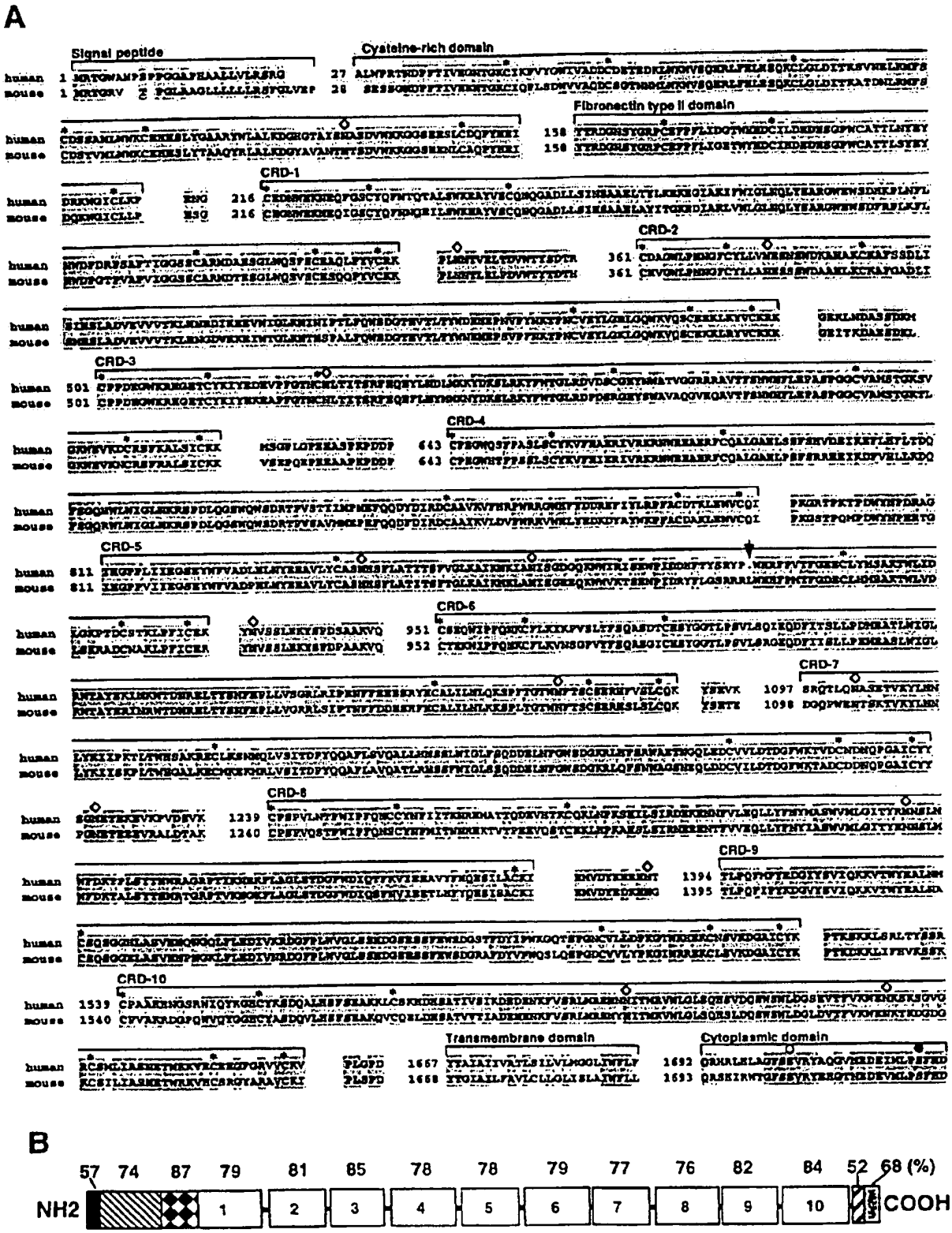
5.0
4.0
3.0
2.0
3.8
3.0

Figure 2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Signal peptide
Cysteine-rich domain
Fibronectin type II domain
CRD-1
CRD-2
CRD-3
CRD-4
CRD-5
CRD-6
CRD-7
CRD-8
CRD-9
CRD-10
Transmembrane domain
Cytoplasmic domain
human
mouse
B
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NH2
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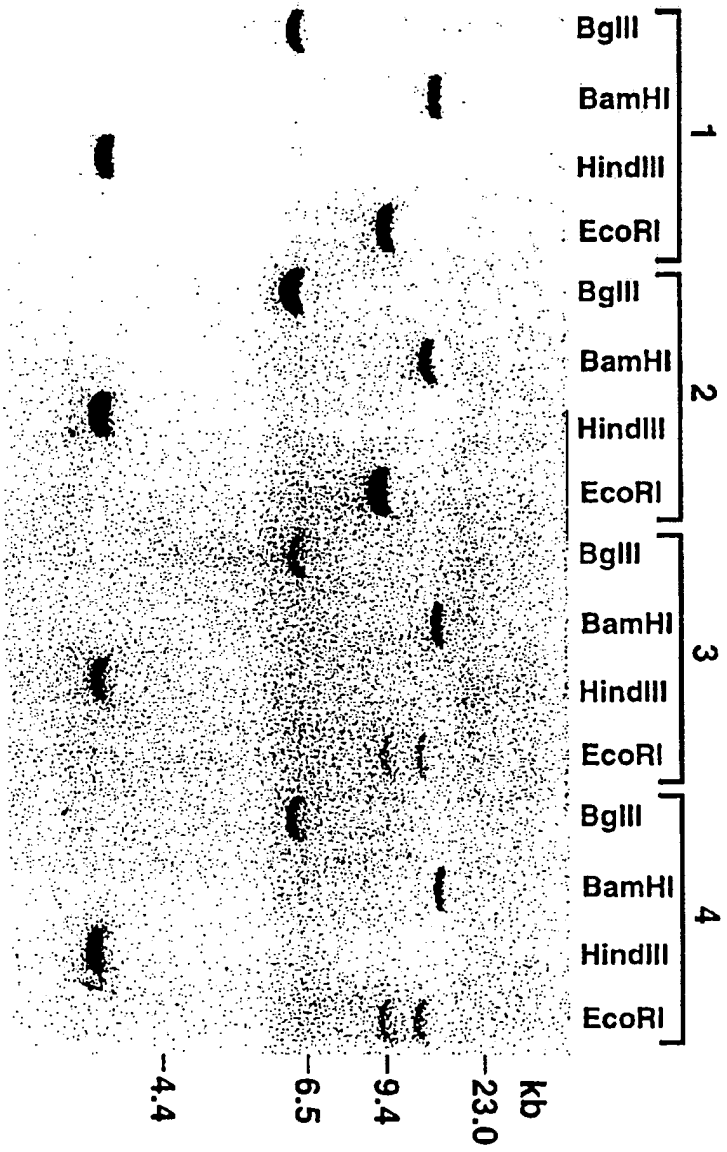
COOH

Figure 3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HindIII
EcoRI
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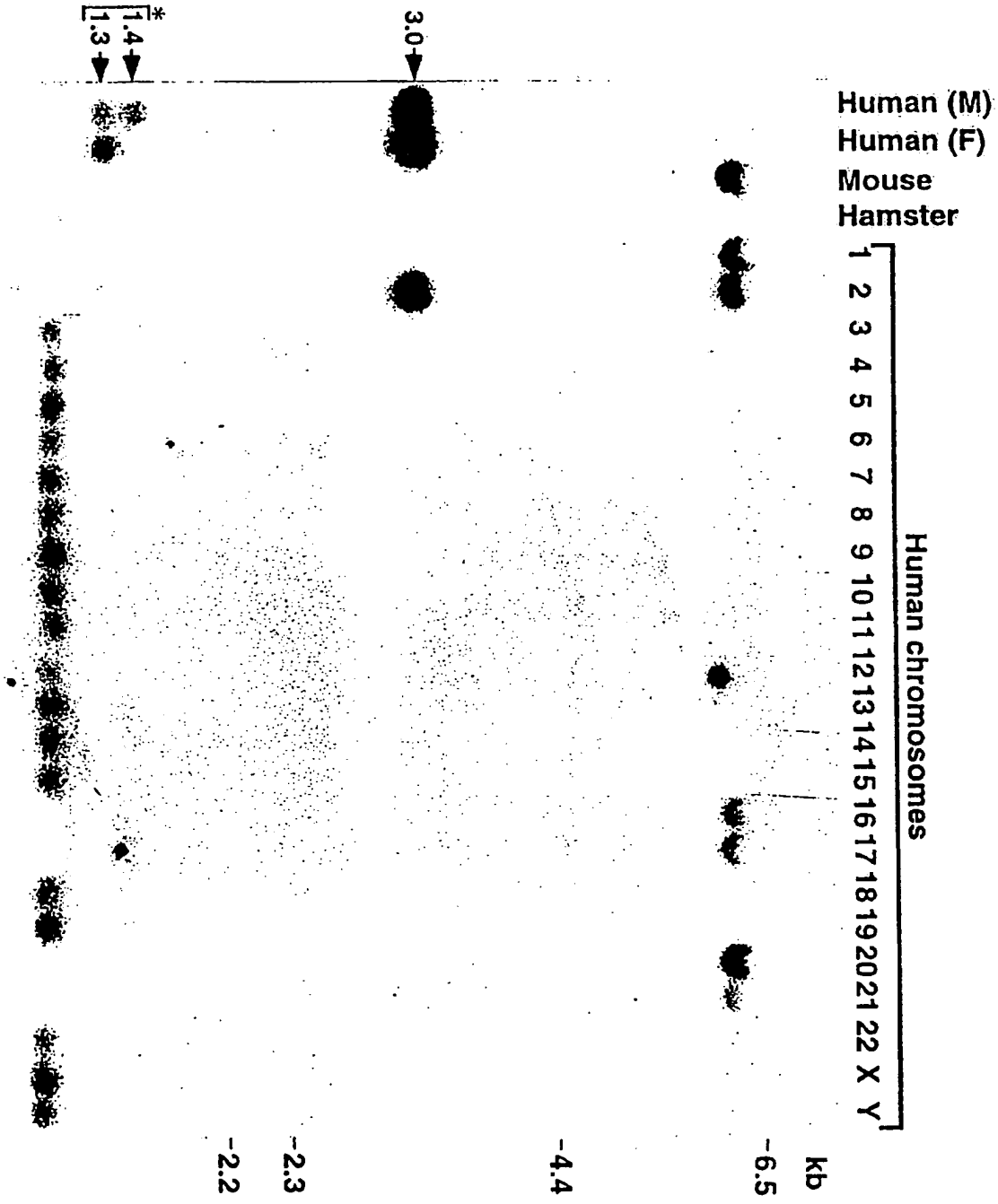
6.5
9.4
23.0
kb

Figure 4


Human (M)
Human (F)
Mouse
Hamster
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
Human chromosomes
kb
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-4.4
-2.3
-2.2
3.0
1.4
1.3
*

Figure 5

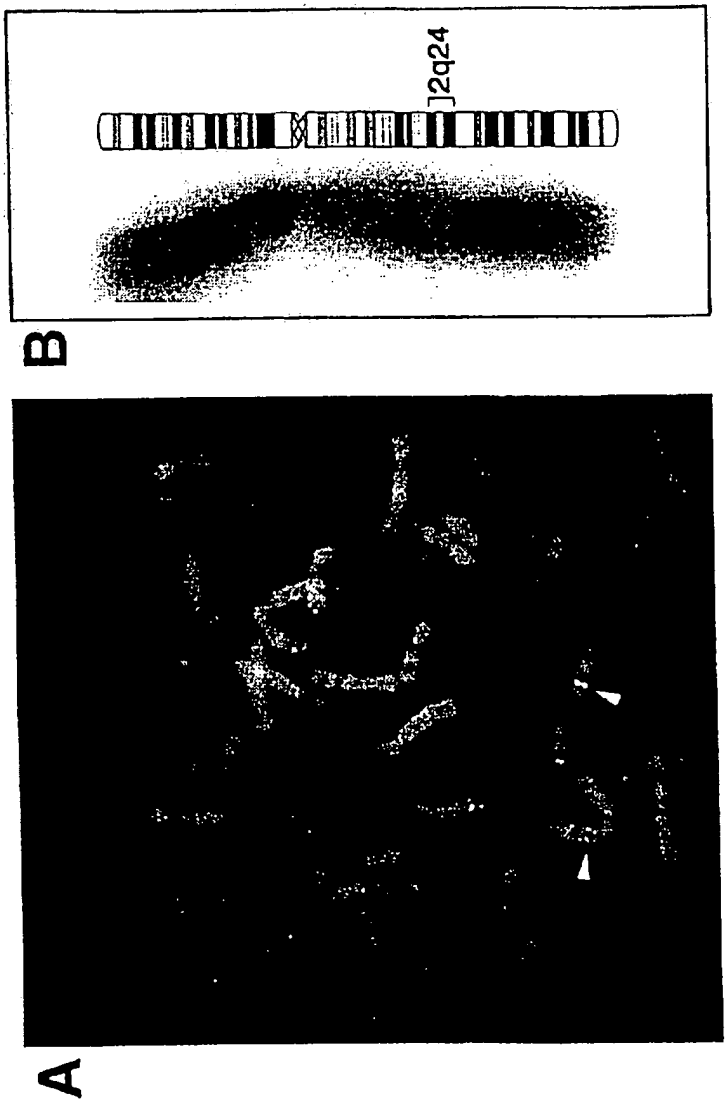
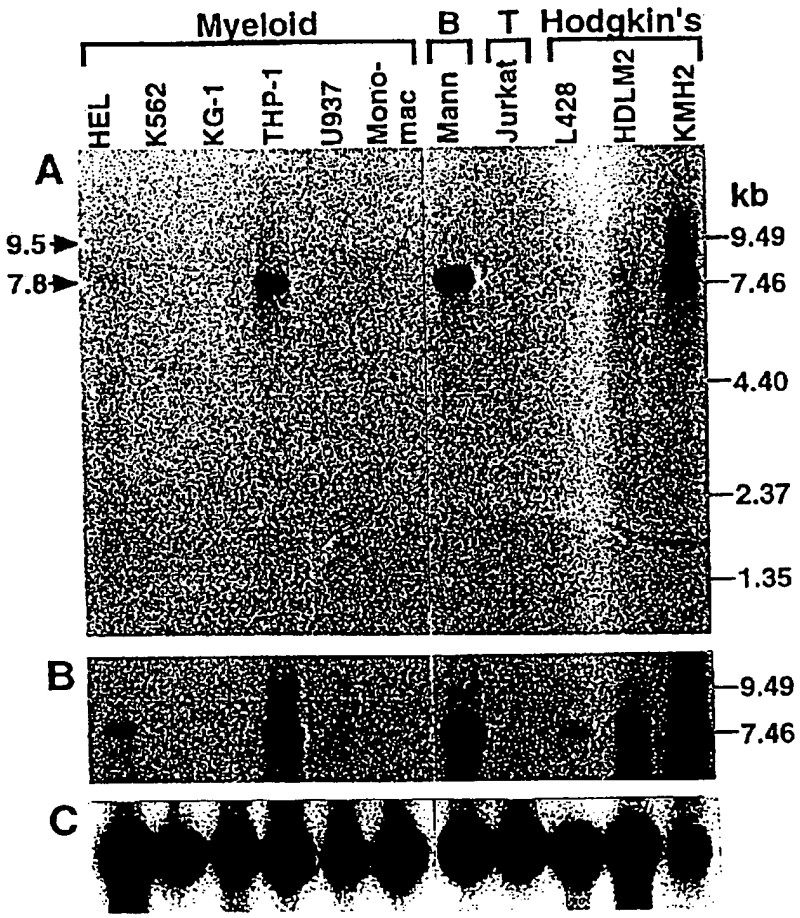


Figure 6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T
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U937
Mono-mac
Mann
Jurkat
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A
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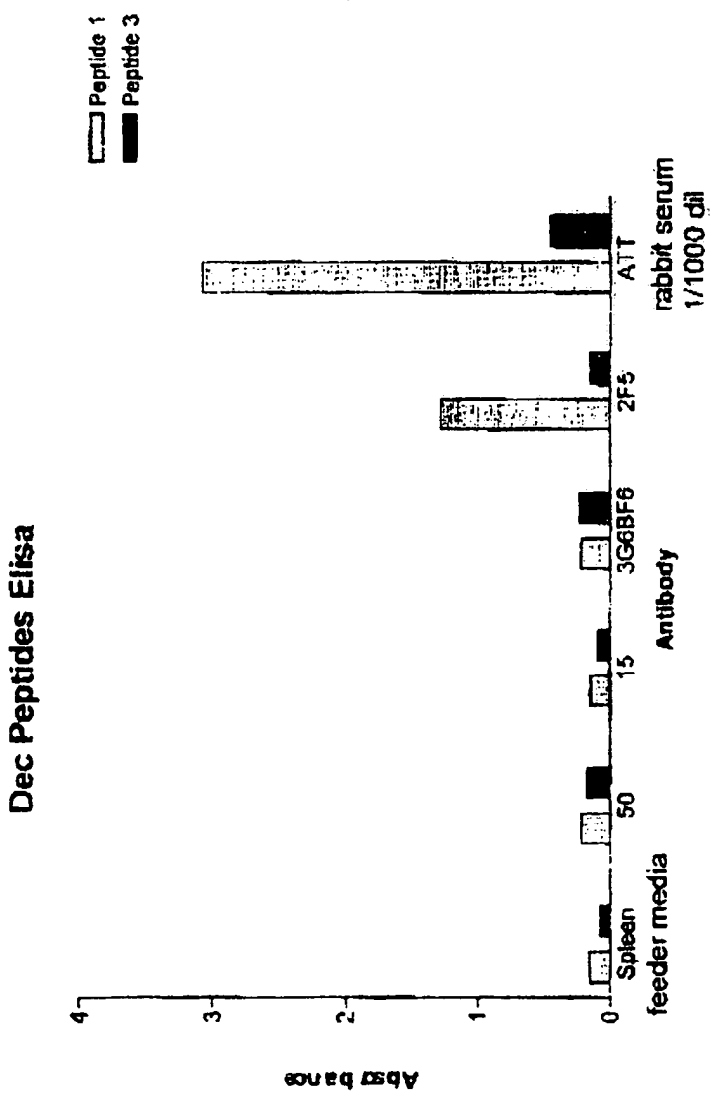
1.35
B
9.49
7.46
C

Figure 7

Figure 8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Peptide 3
Abs/ bkgnd
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3
4
Spleen
feeder media
50
15
3G6BF6
2F5
ATT
Antibody
rabbit serum
1/1000 dil

Figure 9

DEC-205 DNA sequence (coding region only)

1 ATGAGGACAG GCTGGGCGCA CCCCTCGCCG CCCGGCGGGG CTCCTCATGC

51 TGCTCTTCTG GTTCTTCGAT CTCGCGGAGC CCTCTGGCCG CGCACTAATG

101 ACCCCTTCAC CATCGTCCAT GGAAATACGG GCAAGTGCAT CAAGCCAGTG

151 TATGGCTGGA TAGTAGCAGA CGACTGTGAT GAAACTGAGG ACAAGTTATG

201 GAAGTGGGTG TCCCAGCATC GGCTCTTTCA TTTGCACTCC CAAAAGTGCC

251 TTGGCCTCGA TATTACCAAA TCGGTAAATG AGCTGAGAAT GTTCAGCTGT

301 GACTCCAGTG CCATGCTGTG GTGGAAATGT GAGCACCACT CTCTGTACGG

351 AGCTGCCCGG TACTGGCTGG CTCTGAAGGA TGGACATGGC ACAGCAATCT

401 CAAATGCATC TGATGTCTGG AAGAAAGGAG GCTCAGAGGA AAGCCTTTGT

451 GACCAGCCTT ATCATGAGAT CTATACCAGA GATGGGAACT CTTATGGGAG

501 ACCTTGTGAA TTTCCATTCT TAATTGATGG GACCTGGCAT CATGATTGCA

551 TTCTTGATGA AGATCATAGT GGGCCATGGT GTGCCACCAC CTTAAATTAT

601 GAATATGACC GAAAGTGGGG CATCTGCTTA AAGCCTGAAA ACGGTTGTGA

651 AGATAATTGG GAAAAGAACG AGCAGTTTGG AAGTTGCTAC CAATTTAATA

701 CTCAGACGGC TCTTTCTTGG AAAGAAGCTT ATGTTTCATG TCAGAATCAA

751 GGAGCTGATT TACTGAGCAT CAACAGTGCT GCTGAATTAA CTTACCTTAA

801 AGAAAAAGAA GGCATTGCTA AGATTTTCTG GATTGGTTTA AATCAGCTAT

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901 AACTGGGATC CAGACAGGCC CAGTGCACCT ACTATAGGTG GCTCCAGCTG

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1151 AGGCACATGC GAAATGCAAA GCCTTCAGTA GTGACCTAAT CAGCATTCAT

1201 TCTCTAGCAG ATGTGGAGGT GGTTGTCACA AAACTCCATA ATGAGGATAT

1251 CAAAGAAGAA GTGTGGATAG GCCTTAAGAA CATAAACATA CCAACTTTAT

Figure 10

1301 TTCAGTGGTC AGATGGTACT GAAGTTACTC TAACATATTG GGATGAGAAT

1351 GAGCCAAATG TTCCCTACAA TAAGACGCCC AACTGTGTTT CCTACTTAGG

1401 AGAGCTAGGT CAGTGGAAAG TCCAATCATG TGAGGAGAAA CTAAAATATG

1451 TATGCAAGAG AAAGGGAGAA AAACTGAATG ACGCAAGTTC TGATAAGATG

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1601 GCAGATTTGA GCAAGAATAC CTAAATGATT TGATGAAAAA GTATGATAAA

1651 TCTCTAAGAA AATACTTCTG GACTGGCCTG AGAGATGTAG ATTCTTGTGG

1701 AGAGTATAAC TGGGCAACTG TTGGTGGAAG AAGGCGGGCT GTAACCTTTT

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1851 CAAAGCACTT TCAATTTGCA AGAAAATGAG TGGACCCCTT GGGCCTGAAG

1901 AAGCATCCCC TAAGCCTGAT GACCCCTGTC CTGAAGGCTG GCAGAGTTTC

1951 CCCGCAAGTC TTTCTTGTTA TAAGGTATTC CATGCAGAAA GAATTGTAAG

2001 AAAGAGGAAC TGGGAAGAAG CTGAACGATT CTGCCAAGCC CTTGGAGCAC

2051 ACCTTTCTAG CTTCAGCCAT GTGGATGAAA TAAAGGAATT TCTTCACTTT

2101 TTAACGGACC AGTTCAGTGG CCAGCATTGG CTGTGGATTG GTTTGAATAA

2151 AAGGAGCCCA GATTTACAAG GATCCTGGCA ATGGAGTGAT CGTACACCAG

2201 TGTCTACTAT TATCATGCCA AATGAGTTTC AGCAGGATTA TGACATCAGA

2251 GACTGTGCTG CTGTCAAGGT ATTTCATAGG CCATGGCGAA GAGGCTGGCA

2301 TTTCTATGAT GATAGAGAAT TTATTTATTT GAGGCCTTTT GCTTGTGATA

2351 CAAAACTTGA ATGGGTGTGC CAAATTCCAA AAGGCCGTAC TCCAAAAACA

2401 CCAGACTGGT ACAATCCAGA CCGTGCTGGA ATTCATGGAC CTCCACTTAT

2451 AATTGAAGGA AGTGAATATT GGTTTGTTGC TGATCTTCAC CTAAACTATG

2501 AAGAAGCCGT CCTGTACTGT GCCAGCAATC ACAGCTTTCT TGCGACTATA

2551 ACATCTTTTG TGGGACTAAA AGCCATCAAA AACAAAATAG CAAATATATC

2601 TGGTGATGGA CAGAAGTGGT GGATAAGAAT TAGCGAGTGG CCAATAGATG

Figure 10 (cont)

2651 ATCATTTTAC ATACTCACGA TATCCATGGC ACCGCTTTCC TGTGACATTT

2701 GGAGAGGAAT GCTTGTACAT GTCTGCCAAG ACTTGGCTTA TCGACTTAGG

2751 TAAACCAACA GACTGTAGTA CCAAGTTGCC CTTCATCTGT GAAAAATATA

2801 ATGTTTCTTC GTTAGAGAAA TACAGCCCAG ATTCTGCAGC TAAAGTGCAA

2851 TGTTCTGAGC AATGGATTCC TTTTCAGAAT AAGTGTTTTC TAAAGATCAA

2901 ACCCGTGTCT CTCACATTTT CTCAAGCAAG CGATACCTGT CACTCCTATG

2951 GTGGCACCCT TCCTTCAGTG TTGAGCCAGA TTGAACAAGA CTTTATTACA

3001 TCCTTGCTTC CGGATATGGA AGCTACTTTA TGGATTGGTT TGCGCTGGAC

3051 TGCCTATGAA AAGATAAACA AATGGACAGA TAACAGAGAG CTGACGTACA

3101 GTAACTTTCA CCCATTATTG GTTAGTGGGA GGCTGAGAAT ACCAGAAAAT

3151 TTTTTTGAGG AAGAGTCTCG CTACCACTGT GCCCTAATAC TCAACCTCCA

3201 AAAATCACCG TTTACTGGGA CGTGGAATTT TACATCCTGC AGTGAACGCC

3251 ACTTTGTGTC TCTCTGTCAG AAATATTCAG AAGTTAAAAG CAGACAGACG

3301 TTGCAGAATG CTTCAGAAAC TGTAAAGTAT CTAAATAATC TGTACAAAAT

3351 AATCCCAAAG ACTCTGACTT GGCACAGTGC TAAAAGGGAG TGTCTGAAAA

3401 GTAACATGCA GCTGGTGAGC ATCACGGACC CTTACCAGCA GGCATTCCTC

3451 AGTGTGCAGG CGCTCCTTCA CAACTCTTCC TTATGGATCG GACTCTTCAG

3501 TCAAGATGAT GAACTCAACT TTGGTTGGTC AGATGGGAAA CGTCTTCATT

3551 TTAGTCGCTG GGCTGAAACT AATGGGCAAC TCGAAGACTG TGTAGTATTA

3601 GACACTGATG GATTCTGGAA AACAGTTGAT TGCAATGACA ATCAACCAGG

3651 TGCTATTTGC TACTATTCAG GAAATGAGAC TGAAAAAGAG GTCAAACCAG

3701 TTGACAGTGT TAAATGTCCA TCTCCTGTTC TAAATACTCC GTGGATACCA

3751 TTTCAGAACT GTTGCTACAA TTTCATAATA ACAAAGAATA GGCATATGGC

3801 AACAACACAG GATGAAGTTC ATACTAAATG CCAGAAACTG AATCCAAAAT

3851 CACATATTCT GAGTATTCGA GATGAAAAGG AGAATAACTT TGTTCTTGAG

3901 CAACTGCTGT ACTTCAATTA TATGGCTTCA TGGGTCATGT TAGGAATAAC

3951 TTATAGAAAT AATTCTCTTA TGTGGTTTGA TAAGACCCCA CTGTCATATA

Figure 10 (cont)

4001 CACATTGGAG AGCAGGAAGA CCAACTATAA AAAATGAGAA GTTTTTGGCT

4051 GGTTTAAGTA CTGACGGCTT CTGGGATATT CAAACCTTTA AAGTTATTGA

4101 AGAAGCAGTT TATTTTCACC AGCACAGCAT TCTTGCTTGT AAAATTGAAA

4151 TGGTTGACTA CAAAGAAGAA CATAATACTA CACTGCCACA GTTTATGCCA

4201 TATGAAGATG GTATTTACAG TGTTATTCAA AAAAAGGTAA CATGGTATGA

4251 AGCATTAAAC ATGTGTTCTC AAAGTGGAGG TCACTTGGCA AGCGTTCACA

4301 ACCAAAATGG CCAGCTCTTT CTGGAAGATA TTGTAAAACG TGATGGATTT

4351 CCACTATGGG TTGGGCTCTC AAGTCATGAT GGAAGTGAAT CAAGTTTTGA

4401 ATGGTCTGAT GGTAGTACAT TTGACTATAT CCCATGGAAA GGCCAAACAT

4451 CTCCTGGAAA TTGTGTTCTC TTGGATCCAA AAGGAACTTG GAAACATGAA

4501 AAATGCAACT CTGTTAAGGA TGGTGCTATT TGTTATAAAC CTACAAAATC

4551 TAAAAAGCTG TCCCGTCTTA CATATTCATC AAGATGTCCA GCAGCAAAAG

4601 AGAATGGGTC ACGGTGGATC CAGTACAAGG GTCACTGTTA CAAGTCTGAT

4651 CAGGCATTGC ACAGTTTTTC AGAGGCCAAA AAATTGTGTT CAAAACATGA

4701 TCACTCTGCA ACTATCGTTT CCATAAAAGA TGAAGATGAG AATAAATTTG

4751 TGAGCAGACT GATGAGGGAA AATAATAACA TTACCATGAG AGTTTGGCTT

4801 GGATTATCTC AACATTCTGT TGACCAGTCT TGGAGTTGGT TAGATGGATC

4851 AGAAGTGACA TTTGTCAAAT GGGAAAATAA AAGTAAGAGT GGTGTTGGAA

4901 GATGTAGCAT GTTGATAGCT TCAAATGAAA CTTGGAAAAA AGTTGAATGT

4951 GAACATGGTT TTGGAAGAGT TGTCTGCAAA GTGCCTCTGG GCCCTGATTA

5001 CACAGCAATA GCTATCATAG TTGCCACACT AAGTATCTTA GTTCTCATGG

5051 GCGGACTGAT TTGGTTCCTC TTCCAAAGGC ACCGTTTGCA CCTGGCGGGT

5101 TTCTCATCAG TTCGATATGC ACAAGGAGTG AATGAAGATG AGATTATGCT

5151 TCCTTCTTTC CATGAC

Figure 10 (cont)

DEC-205 protein sequence

1 MRTGWAHPSP PGGAPHAALL VLRSRGALWP RTNDPFTIVH GNTGKCIKPV

51 YGWIVADDCD ETEDKLWKWV SQHRLFHLHS QKCLGLDITK SVNELRMFSC

101 DSSAMLWWKC EHHSLYGAAR YWLALKDGHG TAISNASDVW KKGGSEESLC

151 DQPYHEIYTR DGNSYGRPCE FPFLIDGTWH HDCILDEDHS GPWCATTLNY

201 EYDRKWGICL KPENGCEDNW EKNEQFGSCY QFNTQTALSW KEAYVSCQNQ

251 GADLLSINSA AELTYLKEKE GIAKIFWIGL NQLYSARGWE WSDHKPLNFL

301 NWDPDRPSAP TIGGSSCARM DAESGLWQSF SCEAQLPYVC RKPLNNTVEL

351 TDVWTYSDTR CDAGWLPNNG FCYLLVNESN SWDKAHAKCK AFSSDLISIH

401 SLADVEVVVT KLHNEDIKEE VWIGLKNINI PTLFQWSDGT EVTLTYWDEN

451 EPNVPYNKTP NCVSYLGELG QWKVQSCEEK LKYVCKRKGE KLNDASSDKM

501 CPPDEGWKRH GETCYKIYED EVPFGTNCNL TITSRFEQEY LNDLMKKYDK

551 SLRKYFWTGL RDVDSCGEYN WATVGGRRRA VTFSNWNFLE PASPGGCVAM

601 STGKSVGKWE VKDCRSFKAL SICKKMSGPL GPEEASPKPD DPCPEGWQSF

651 PASLSCYKVF HAERIVRKRN WEEAERFCQA LGAHLSSFSH VDEIKEFLHF

701 LTDQFSGQHW LWIGLNKRSP DLQGSWQWSD RTPVSTIIMP NEFQQDYDIR

751 DCAAVKVFHR PWRRGWHFYD DREFIYLRPF ACDTKLEWVC QIPKGRTPKT

801 PDWYNPDRAG IHGPPLIIEG SEYWFVADLH LNYEEAVLYC ASNHSFLATI

851 TSFVGLKAIK NKIANISGDG QKWWIRISEW PIDDHFTYSR YPWHRFPVTF

901 GEECLYMSAK TWLIDLGKPT DCSTKLPFIC EKYNVSSLEK YSPDSAAKVQ

951 CSEQWIPFQN KCFLKIKPVS LTFSQASDTC HSYGGTLPSV LSQIEQDFIT

1001 SLLPDMEATL WIGLRWTAYE KINKWTDNRE LTYSNFHPLL VSGRLRIPEN

1051 FFEEESRYHC ALILNLQKSP FTGTWNFTSC SERHFVSLCQ KYSEVKSRQT

1101 LQNASETVKY LNNLYKIIPK TLTWHSAKRE CLKSNMQLVS ITDPYQQAFL

1151 SVQALLHNSS LWIGLFSQDD ELNFGWSDGK RLHFSRWAET NGQLEDCVVL

1201 DTDGFWKTVD CNDNQPGAIC YYSGNETEKE VKPVDSVKCP SPVLNTPWIP

1251 FQNCCYNFII TKNRHMATTQ DEVHTKCQKL NPKSHILSIR DEKENNFVLE

Figure 11

1301 QLLYFNYMAS WVMLGITYRN NSLMWFDKTP LSYTHWRAGR PTIKNEKFLA

1351 GLSTDGFWDI QTFKVIEEAV YFHQHSILAC KIEMVDYKEE HNTTLPQFMP

1401 YEDGIYSVIQ KKVTWYEALN MCSQSGGHLA SVHNQNGQLF LEDIVKRDGF

1451 PLWVGLSSHD GSESSFEWSD GSTFDYIPWK GQTSPGNCVL LDPKGTWKHE

1501 KCNSVKDGAI CYKPTKSKKL SRLTYSSRCP AAKENGSRWI QYKGHCYKSD

1551 QALHSFSEAK KLCSKHDHSA TIVSIKDEDE NKFVSRLMRE NNNITMRVWL

1601 GLSQHSVDQS WSWLDGSEVT FVKWENKSKS GVGRCSMLIA SNETWKKVEC

1651 EHGFGRVVCK VPLGPDYTAI AIIVATLSIL VLMGGLIWFL FQRHRLHLAG

1701 FSSVRYAQGV NEDEIMLPSF HD*

Figure 11 (cont)

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/NZ 97/00068

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C07K 14/74, 16/28; C12N 15/12; A61K 38/17, 39/395, 39/385; G01N 33/566

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
SEE ELECTRONIC DATABASES BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
SEE ELECTRONIC DATABASES BELOW

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT-dendrit:, receptor#, antibod:, anti()bod:, immunoglobulin#; MEDLINE - DEC205, DEC()205; STN (DGENE), GENBANK, SWISS-PROT,PIR,EMBO - sequence search as in claim 1, Figure 11

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AU A 49702/96 (The Rockfeller University) 8 August 1996 See entire document	1-22
Y	CELLULAR IMMUNOLOGY (1995) volume 165, pages 302-311 by Swiggard WJ et al. "DEC-205, a 205-kDa protein abundant on mouse dendritic cells and thymic epithelium thet is detected by the monoclonal antibody NLDC-145: Purification, characterisation and N-terminal amino acid sequence" See entire document	14-18, 21, 22

☐ Further documents are listed in the continuation of Box C

☒ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
15 August 1997

Date of mailing of the international search report
09 SEP 1997

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/NZ 97/00068

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 14, 15, 16 (partially)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
they refer to ligands which encompasses a broad range of compounds, and it is not possible or economically viable for the international search to cover the entire subject matter to which the claims are directed.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International Application No.
PCT/NZ 97/00068

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member	
AU	49702/96	WO	96/23882

END OF ANNEX